



# I U C L I D

## D a t a S e t

Existing Chemical ID: 71-43-2  
CAS No. 71-43-2  
EINECS Name benzene  
EC No. 200-753-7  
TSCA Name Benzene  
Molecular Formula C6H6

Rapporteur: Bundesanstalt fuer Arbeitsschutz - BAuA  
Creation date: 12-DEC-1996

Memo: EU Existing Chemicals Programme in the context of Council  
Regulation (EEC) No. 793/93

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Flags (profile): Flags: without flag, confidential, non confidential, WGK  
(DE), TA-Luft (DE), Material Safety Dataset, Risk  
Assessment, Directive 67/548/EEC, SIDS

**1.0.1 Applicant and Company Information**

**Type:** cooperating company  
**Name:** ACNA C.O. in Liquidazione  
**Street:** Piazza della Vittoria, 10  
**Town:** 17056 Cangio (Savona)  
**Country:** Italy

**Source:** German rapporteur  
24-SEP-2002

**Type:** cooperating company  
**Name:** AGIP PETROLI  
**Contact Person:** Dott. ssa F. Iobbi **Date:** 03-JUN-1994  
**Street:** Spacifiche e Qua ita Prodotti, Via Laurentina, 449  
**Town:** 00142 Roma  
**Country:** Italy

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** ALUSUISSE ITALIA SPA  
**Contact Person:** Marco Austoni **Date:** 03-JUN-1994  
**Street:** Divisione Chimica, Stabilimento Ftalital, Via Enrico Fermi, 51  
**Town:** 24020 Scanzorosciate  
**Country:** Italy  
**Phone:** 39-35-652111  
**Telefax:** 39-35-655889

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris la Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** ARAL AG  
**Contact Person:** Mr H.-J. Gorus **Date:** 03-JUN-1994  
**Street:** Dpt CHV, Wittener Str. 45  
**Town:** 44776 Bochum  
**Country:** Germany

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** BASF AG  
**Contact Person:** Mr R. Kotkamp **Date:** 03-JUN-1994  
**Street:** RCP-M 300, Carl-Bosch-Straße 38  
**Town:** 67056 Ludwigshafen  
**Country:** Germany  
**Phone:** 00496216044703  
**Telefax:** 00496216044711  
**Telex:** 464990

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris la Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** Bayer AG  
**Street:** Geb. D8  
**Town:** 51368 Leverkusen  
**Country:** Germany

**Source:** German rapporteur

01-AUG-2000

**Type:** cooperating company  
**Name:** BITMAC LIMITED  
**Contact Person:** Dr D. Whittle **Date:** 03-JUN-1994  
**Street:** Meridian House, Normanby Road  
**Town:** DN15 8QX Scunthorpe, South Humberside  
**Country:** United Kingdom  
**Phone:** 44 724 281555  
**Telefax:** 44 724 281343

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** BP Chemicals LTD  
**Contact Person:** Mr. S.D. Williams **Date:**  
**Street:** Buckingham Palace Road 76  
**Town:** SW1 WOSU LONDON  
**Country:** United Kingdom  
**Phone:** +44-1715811388  
**Telefax:** +44-1715816459

**Source:** BOREALIS POLYMERS OY PORVOO  
BRENNTAG AG Mühlheim an der Ruhr

16-DEC-2004

**Type:** cooperating company  
**Name:** BRENNTAG  
**Street:** Humboldttring 15  
**Town:** 45472 Mühlheim a.d. Ruhr  
**Country:** Germany

**Source:** Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** CEPESA  
**Contact Person:** Mr J.I. Sanchez **Date:** 03-JUN-1994  
**Street:** Avenida de America 32  
**Town:** 28028 Madrid  
**Country:** Spain

**Source:** BP Chemicals Ltd LONDON

01-AUG-2000 Deutsche Shell Chemie GmbH Eschborn

**Type:** cooperating company  
**Name:** Compañía Española de Petroleos CEPSA  
**Contact Person:** German Burriel Lluna **Date:**  
**Street:** Avda Partenon 12  
**Town:** 28042 Madrid  
**Country:** Spain  
**Phone:** 34913376000  
**Telefax:** 34913376654

**Source:** Compañía Española de Petroleos CEPSA Madrid  
 02-DEC-1998

**Type:** cooperating company  
**Name:** Conoco Limited  
**Contact Person:** D M Haddrill **Date:**  
**Street:** Humber Refinery, South Killingholme  
**Town:** Grimsby South Humberside  
**Country:** United Kingdom  
**Phone:** 0469 571571  
**Telefax:** 0469 555674  
**Telex:** 527111

**Source:** BP Chemicals Ltd LONDON  
 Conoco Limited Warwick  
 Deutsche Shell Chemie GmbH Eschborn  
 Elf Atochem Paris la Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** DEA MINERALOEL AG  
**Contact Person:** Mr W. Linder **Date:** 03-JUN-1994  
**Street:** Dept UWR, Überseering 40  
**Town:** 22297 Hamburg  
**Country:** Germany  
**Phone:** 494063752232  
**Telefax:** 494063753520

**Source:** BP Chemicals Ltd LONDON  
 Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** lead organisation  
**Name:** DEUTSCHE SHELL CHEMIE GMBH  
**Contact Person:** Dr S. Trebert-Haeberlin **Date:** 03-JUN-1994  
**Street:** Koelner Strasse 6  
**Town:** 65727 Eschborn  
**Country:** Germany  
**Phone:** 00496196474245  
**Telefax:** 00496196474502

**Source:** BP Chemicals Ltd LONDON  
 Deutsche Shell Chemie GmbH Eschborn  
 Elf Atochem Paris la Defense 10

16-DEC-2004

**Type:** cooperating company  
**Name:** DOW BENELUX N.V.  
**Contact Person:** Dr J. Wilmer **Date:** 03-JUN-1994  
**Street:** Herbert H. Dowweg 5, P.O. Box 48  
**Town:** 4530 AA Terneuzen  
**Country:** Netherlands  
**Phone:** 41-1-728 2996  
**Telefax:** 41-1-728 2965

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** Dow Europe S.A.  
**Contact Person:** Dr. J. Wilmer **Date:** 06-MAY-1994  
**Street:** Bachtobelstrasse 3  
**Town:** CH-8810 Horgen  
**Country:** Switzerland  
**Phone:** +41-(0)1-728 2996  
**Telefax:** +41-(0)1-728 2965

**Remark:** Please send all correspondence to Dr. J. Wilmer.  
**Source:** Dow Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** DSM  
**Contact Person:** Dr J. Neis **Date:** 03-JUN-1994  
**Street:** Corporate Safety, Env. Health & Technology, P.O. Box 6500  
**Town:** 6401 JH Heerlen  
**Country:** Netherlands  
**Phone:** 003144788111  
**Telefax:** 003145787112  
**Telex:** 56018

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** EC ERDÖLCHEMIE  
**Contact Person:** Dr B. Richter **Date:** 03-JUN-1994  
**Street:** Produktionsabteilung VII SUU, Alte Strasse 201  
**Town:** 50769 Köln  
**Country:** Germany  
**Phone:** 00492133551  
**Telefax:** 00492133555789  
**Telex:** 8517361

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris la Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** ELF ATOCHEM SA  
**Contact Person:** Mr J. Grevin **Date:** 03-JUN-1994  
**Street:** Div. Petrochimie, 4, Cours Michelet, La Defense  
**Town:** 92997 Paris  
**Country:** France  
**Phone:** 0033149007109  
**Telefax:** 0033149007214  
**Telex:** 042620750

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** ENI SpA divisione refining e marketing  
**Contact Person:** Fiorella IOBBI **Date:** 02-JAN-2003  
**Street:** Laurentina 449  
**Town:** 00142 ROMA RM  
**Country:** Italy  
**Phone:** +39-06-59886394  
**Telefax:** +39-06-59886736  
**Telex:** 614031 I

**Source:** ENI SpA - Divisione Refining & Marketing ROMA

30-MAY-2003

**Type:** cooperating company  
**Name:** ENICHEM AUGUSTA INDUSTRIALE  
**Contact Person:** Mrs D. Calcinai **Date:** 03-JUN-1994  
**Street:** Via Medici del Vascello 26  
**Town:** 20138 Milan  
**Country:** Italy  
**Phone:** 003925202904  
**Telefax:** 0039252029824  
**Telex:** 310246

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** ENICHEM ELASTOMERS LTD  
**Contact Person:** Mr J. Harris **Date:** 03-JUN-1994  
**Street:** Charleston Road  
**Town:** SO4 6YY Hythe, Southampton  
**Country:** United Kingdom  
**Phone:** 0044703894919  
**Telefax:** 0044703891655  
**Telex:** 47519

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** ENICHEM SPA  
**Contact Person:** Ing. L. Peres **Date:** 03-JUN-1994  
**Street:** Piazza Boldrini 1  
**Town:** 20097 San Donato Milanese  
**Country:** Italy  
**Phone:** 003925202904  
**Telefax:** 0039252029824  
**Telex:** 310246

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** ENICHEM SYNTHESIS SPA  
**Contact Person:** Dr P. Novero **Date:** 03-JUN-1994  
**Street:** Via Medici del Vascello 40/B  
**Town:** 20138 Milano  
**Country:** Italy  
**Phone:** 39-2-5203-9273  
**Telefax:** 39-2-5203-9827

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** EXXON CHEMICAL HOLLAND  
**Contact Person:** Mr L. Van Dijk **Date:** 03-JUN-1994  
**Street:** Merwedeweg 21, Havennummer 5625, Postbus 1322  
**Town:** 3180 AH Rozenburg  
**Country:** Netherlands  
**Phone:** 0031181955600  
**Telefax:** 0031181955892

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** FINA-BOREALIS NV  
**Contact Person:** R. Pottie **Date:** 03-JUN-1994  
**Street:** Schelde Laan, 10  
**Town:** 2030 Antwerpen  
**Country:** Belgium  
**Phone:** 32-3-545-2470  
**Telefax:** 32-3-545-2456

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** ICI CHEMICALS & POLYMERS LIMITED  
**Contact Person:** Mr M.G. Penman **Date:** 03-JUN-1994  
**Street:** PO Box 14, The Heath  
**Town:** WA7 4QF Runcorn, Cheshire  
**Country:** United Kingdom

**Remark:** Contact name and address:  
Mr M.G. Penman  
Occupational Health  
ICI Chemicals & Polymers Ltd  
P.O. Box 54  
Wilton  
Middlesbrough  
Cleveland  
TS90 8JA

Telephone No.: 0642 454144  
Telex No. : 94028500 ICIC G  
Telefax No. : 0642 43370  
**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
ICI Chemicals & Polymers Limited Runcorn, Cheshire

01-AUG-2000

**Type:** cooperating company  
**Name:** KUWAIT RAFFINAZIONE & CHIMICA  
**Contact Person:** P. Crespina **Date:** 03-JUN-1994  
**Street:** Via Nuova delle Brecce, 205  
**Town:** 80147 Napoli  
**Country:** Italy  
**Telefax:** 39-6-5208-8724

**Remark:** Contact name and address:  
P. Crespina  
Kuwait Petroleum Italia  
Viale Dell'Oceano Indiano, 13  
00144 Rome  
Italy

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Kuwait Raffinazione e Chimica Napoli

01-AUG-2000

**Type:** cooperating company  
**Name:** NESTE  
**Contact Person:** Mr S. Loikkanen **Date:** 03-JUN-1994  
**Street:** Neste Environment & Safety, P.O. Box 320  
**Town:** 06101 Porvoo  
**Country:** Finland  
**Phone:** 0035804504483  
**Telefax:** 0035804504781

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** PCK AG Schwedt  
**Street:** Passower Chaussee  
**Town:** 16303 Schwedt/Oder  
**Country:** Germany

**Source:** German rapporteur  
01-AUG-2000

**Type:** cooperating company  
**Name:** PETROFINA SA  
**Contact Person:** J.Ph. Gennart **Date:** 03-JUN-1994  
**Street:** Rue de l'Industrie, 52  
**Town:** 1040 Brussels  
**Country:** Belgium  
**Phone:** 32-2-288-9111  
**Telefax:** 32-2-288-3334

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** Petroleos de Portugal - Petrogal, S.A.  
**Street:** Rua das Flores 7  
**Town:** 1200 Lisboa  
**Country:** Portugal

**Source:** German rapporteur  
01-AUG-2000

**Type:** cooperating company  
**Name:** POLIOLI SPA  
**Contact Person:** Dr E. Nerci **Date:** 03-JUN-1994  
**Street:** Viale Emilia, 85  
**Town:** 20093 Cologno Monzese, Milan  
**Country:** Italy  
**Phone:** 39-35-993900  
**Telefax:** 39-35-492849

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** PRAOIL S.R.L.  
**Contact Person:** L. Biasin **Date:** 03-JUN-1994  
**Street:** Milanofiori, Strada 2, Palazzo F7  
**Town:** 20090 Assago  
**Country:** Italy  
**Phone:** 0039252026985  
**Telefax:** 0039252026986  
**Telex:** 331625

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** other: company  
**Name:** REPSOL PETROLEO S.A.  
**Contact Person:** Rogelio Martínez de Azagra **Date:** 27-MAY-1994  
**Street:** CASTELLANA, 278-280  
**Town:** 28046 MADRID  
**Country:** Spain  
**Phone:** 91-3488100/3488000  
**Telefax:** 91-3142821/3489494  
**Telex:** 49840

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996

**Type:** cooperating company  
**Name:** RHONE POULENC CHEMICALS LIMITED  
**Contact Person:** D. Philipe **Date:** 03-JUN-1994  
**Street:** Oak House, Reeds Crescent  
**Town:** WD1 1QH Watford, Hertfordshire  
**Country:** United Kingdom  
**Phone:** 33-72-73-6712  
**Telefax:** 33-72-73-6782

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** RHONE-POULENC CHEMICALS  
**Contact Person:** Mr A.J. Steel **Date:** 03-JUN-1994  
**Street:** Commercial Director, Staveley Chemicals Ltd  
**Town:** S43 2PB Chesterfield, Derbyshire  
**Country:** United Kingdom  
**Phone:** 33-72-736712  
**Telefax:** 33-72-736782

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

01-AUG-2000

**Type:** cooperating company  
**Name:** Ruetgers-Vft AG  
**Contact Person:** Dr R. Thoms **Date:** 03-JUN-1994  
**Street:** Postfach 12 05 52  
**Town:** 47125 Duisburg  
**Country:** Germany

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10

01-AUG-2000

**Type:** cooperating company  
**Name:** Ruhr Oel GmbH  
**Street:** Alexander-von-Humboldt-Straße  
**Town:** 45896 Gelsenkirchen-Hassel  
**Country:** Germany

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** SHELL CHEMICAL INTERNATIONAL TRADING COMPANY  
**Contact Person:** Mr B. Reeve **Date:** 03-JUN-1994  
**Street:** Shell Centre  
**Town:** SE1 7PG London  
**Country:** United Kingdom  
**Phone:** 0044719345209  
**Telefax:** 0044719344234

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** SHELL CHEMICALS UK LTD  
**Contact Person:** Miss J. Eve **Date:** 03-JUN-1994  
**Street:** Heronbridge House, Chester Business Park  
**Town:** CH4 9QA Chester  
**Country:** United Kingdom  
**Phone:** 0044244685000  
**Telefax:** 0044244685010  
**Telex:** 21795

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** SHELL CHIMIE  
**Street:** 89 Bd Franklin Roosevelt  
**Town:** 92564 Rueil Malmaison  
**Country:** France  
**Phone:** 33 1 47 14 71 00  
**Telefax:** 33 1 47 14 74 67  
**Telex:** SHELL 615013F

**Source:** SHELL CHIMIE Rueil Malmaison  
SHELL FRANCE Rueil Malmaison  
01-AUG-2000

**Type:** cooperating company  
**Name:** SHELL INTERNATIONAL CHEMICAL COMPANY  
**Contact Person:** Dr P. Brocklehurst **Date:** 03-JUN-1994  
**Street:** Shell Centre  
**Town:** SEA 7PG London  
**Country:** United Kingdom

**Phone:** 0044719345209  
**Telefax:** 0044719344234

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** Shell Nederland Chemie B.V.  
**Contact Person:** Ir. H. Jolie **Date:**  
**Street:** Vondelingenweg 601  
**Town:** 3196 KK Eschborn Rotterdam (Pernis)  
**Country:** Netherlands  
**Phone:** +31.(0)10.2317005  
**Telefax:** +31.(0)10.2317125  
**Telex:** 30502 NL

**Source:** Shell Nederland Chemie B.V. Rotterdam (Pernis)  
13-DEC-1996

**Type:** cooperating company  
**Name:** SHELL UK LIMITED  
**Contact Person:** M.J.E. Brain **Date:** 03-JUN-1994  
**Street:** Shell Mex House, The Strand  
**Town:** WC2R 0DX London  
**Country:** United Kingdom  
**Phone:** 44-71-257-3522  
**Telefax:** 44-71-257-3212

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Shell UK Ltd. London  
01-AUG-2000

**Type:** cooperating company  
**Name:** SSAB Tunnplat AB  
**Town:** 97188 Lulea  
**Country:** Sweden

**Source:** German rapporteur  
01-AUG-2000

**Type:** cooperating company  
**Name:** SÄCHSISCHE OLEFINWERKE AG  
**Contact Person:** Mr Heyner/Fischer **Date:** 03-JUN-1994  
**Street:** Werkstraße  
**Town:** 7202 Böhlen  
**Country:** Germany

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10  
01-AUG-2000

**Type:** cooperating company  
**Name:** TOTAL-RAFFINAGE DISTRIBUTION  
**Contact Person:** Mr P. Carpentier **Date:** 03-JUN-1994  
**Street:** Petrochemicals Distribution, La Defense  
**Town:** 92069 Paris  
**Country:** France  
**Phone:** 33141352722  
**Telefax:** 33141352705  
**Cedex:** 47

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** WINTERSHALL AG  
**Contact Person:** Mr Jaegers Kuepper **Date:** 03-JUN-1994  
**Street:** Postfach 104020  
**Town:** 34112 Kassel  
**Country:** Germany  
**Phone:** 49-561-301-1059  
**Telefax:** 49-561-301-1027

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** ZAKLADY CHEMICZNE BLACHOWNIA  
**Contact Person:** Mr T. Bek **Date:** 03-JUN-1994  
**Street:** 47-225 Kedzierzyn-Kozle  
**Country:** other

**Country:** Poland  
**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
01-AUG-2000

**Type:** cooperating company  
**Name:** ÖMV-Chemie Linz GmbH  
**Street:** St. Peterstrasse 25  
**Town:** 4021 Linz  
**Country:** Austria

**Source:** German rapporteur  
01-AUG-2000

## 1.2 Synonyms and Tradenames

Annulene

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

Elf Atochem Paris La Defense 10  
Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
Ruetgers-Vft AG Duisburg  
TOTAL PARIS LA DEFENSE

01-AUG-2000

Benzen

**Source:** Sächsische Olefinwerke GmbH Böhlen  
13-DEC-1996

Benzene

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
BRENNTAG AG Mülheim an der Ruhr  
Deutsche Shell Chemie GmbH Eschborn  
EXXON CHEMICAL HOLLAND BV Botlek Rt

01-AUG-2000

Benzene (8CI, 9CI)

**Source:** BASF AG Ludwigshafen  
09-DEC-1992

Benzol

**Source:** ACNA C.O. in Liquidazione Cengio (Savona)  
Aral Aktiengesellschaft Bochum  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
DEA Mineraloel AG Hamburg  
Deutsche Shell Chemie GmbH Eschborn  
Dow Benelux N.V. AA Terneuzen  
EC Erdölchemie GmbH Köln  
Elf Atochem Paris La Defense 10  
Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
FINA-BOREALIS Antwerpen  
ICI Chemicals & Polymers Limited Runcorn, Cheshire  
PCK AG Schwedt Schwedt/Oder  
REPSOL PETROLEO, S.A. Madrid  
Ruetgers-Vft AG Duisburg  
Shell Chimie Rueil Malmaison  
Shell Nederland Chemie B.V. Rotterdam (Pernis)  
Shell UK Limited, London  
Total Paris La Defense  
ÖMV - Chemie Linz GMBH Linz  
ÖMV Deutschland GmbH Burghausen

13-DEC-1996

## Benzol(e) Phenyl Hydride

**Source:** Rhone-Poulenc Chemicals LTD WATFORD, HERTFORSHIRE  
01-AUG-2000

## Benzole

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
Dow Benelux N.V. AA Terneuzen  
ICI Chemicals & Polymers Limited Runcorn, Cheshire  
REPSOL PETROLEO, S.A. MADRID

01-AUG-2000

## Benzolene

**Source:** Dow Benelux N.V. AA Terneuzen  
REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

## Benzolo

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Elf Atochem Paris La Defense 10  
ENI SpA - Divisione Refining & Marketing ROMA  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
POLIOLI S.P.A. Cologno Monzese (MI)  
PRAOIL S.R.L. ASSAGO MI  
Ruetgers-Vft AG Duisburg  
TOTAL PARIS LA DEFENSE

01-AUG-2000

## Carbon oil

**Source:** Dow Benelux N.V. AA Terneuzen  
REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

## Cicloesatriene

**Source:** POLIOLI S.P.A. Cologno Monzese (MI)

01-AUG-2000

## Coal naphtha

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Dow Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10

Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
REPSOL PETROLEO, S.A. Madrid  
Ruetgers-Vft AG Duisburg  
TOTAL PARIS LA DEFENSE

21-DEC-2004

Cyclohexatrien

**Source:** Sächsische Olefinwerke GmbH Böhlen  
13-DEC-1996

Cyclohexatriene

**Source:** ACNA C.O. in Liquidazione Cengio (Savona)  
Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Dow Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10  
Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
REPSOL PETROLEO, S.A. Madrid  
Rhone-Poulenc Chemicals LTD WATFORD, HERTFORSHIRE  
Ruetgers-Vft AG Duisburg  
Shell Chimie Rueil Malmaison  
Shell Nederland Chemie B.V. Rotterdam (Pernis)  
Shell UK Limited, London  
Total Paris La Defense

01-AUG-2000

Fenilidrine

**Source:** POLIOLI S.P.A. Cologno Monzese (MI)  
01-AUG-2000

Mineral naphtha

**Source:** Enichem Synthesis Milan  
ENICHEM SYNTHESIS S.p.A. MILANO

02-AUG-2000

Phene

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Dow Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
Ruetgers-Vft AG Duisburg

13-DEC-1996 TOTAL PARIS LA DEFENSE

Phenylhydride

**Source:** ACNA C.O. in Liquidazione Cengio (Savona)  
Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Deutsche Shell Chemie GmbH Eschborn  
DOW Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10  
Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
Ruetgers-Vft AG Duisburg  
SHELL CHIMIE RUEIL MALMAISON  
Shell Nederland Chemie B.V. Rotterdam (Pernis)  
Shell UK Limited, London  
Total Paris La Defense

01-AUG-2000

Phenylwasserstoff

**Source:** DEA Mineraloel AG Hamburg  
Sächsische Olefinwerke GmbH Böhlen

01-AUG-2000

Pirobenzolo

**Source:** POLIOLI S.P.A. Cologno Monzese (MI)

01-AUG-2000

Pyrobenzol

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
Aral Aktiengesellschaft Bochum  
BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen  
Bitmac Limited Scunthorpe  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
Dow Benelux N.V. AA Terneuzen  
Elf Atochem Paris La Defense 10  
Enichem S.p.A. Milan  
EXXON CHEMICAL HOLLAND BV Botlek Rt  
Ruetgers-Vft AG Duisburg  
Total Paris La Defense

21-DEC-2004

## Pyrobenzole

**Source:** Aral Aktiengesellschaft Bochum  
 BASF AG Ludwigshafen  
 BASF Antwerpen N. V. Antwerpen 4  
 Bitmac Limited Scunthorpe  
 BP Chemicals Ltd LONDON  
 Deutsche Shell Chemie GmbH Eschborn  
 Dow Benelux N.V. AA Terneuzen  
 Elf Atochem Paris La Defense 10  
 Ruetgers-Vft AG Duisburg  
 TOTAL PARIS LA DEFENSE

13-DEC-1996

Q9111

**Source:** Shell UK Limited, London

13-DEC-1996

## Reinbenzol

**Source:** BRENNTAG AG Mühlheim an der Ruhr  
 PCK AG Schwedt Schwedt/Oder

01-AUG-2000

**1.6.1 Labelling**

**Labelling:** as in Directive 67/548/EEC

**Symbols:** (F) highly flammable  
 (T) toxic

**Nota:** (E) For substances ascribed Nota E the risk phrases R20, R22 to R28 and all combinations of these risk phrases shall be preceded by the word 'also'. E.g. R23 'also' toxic by inhalation

**R-Phrases:** (45) May cause cancer  
 (46) May cause heritable genetic damage  
 (11) Highly flammable  
 (36/38) Irritating to eyes and skin  
 (48/23/24/25) Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed

**S-Phrases:** (65) Harmful: may cause lung damage is swallowed  
 (53) Avoid exposure - obtain special instructions before use  
 (45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

**Source:** German Rapporteur

21-DEC-2004

**Labelling:** provisionally by manufacturer/importer

**S-Phrases:** (16) Keep away from sources of ignition - No smoking

**Source:** BASF AG Ludwigshafen  
 BASF Antwerpen N. V. Antwerpen 4

03-AUG-2000

(73)

### **1.6.2 Classification**

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** carcinogenic, category 1  
**R-Phrases:** (45) May cause cancer

**Source:** German Rapporteur  
21-DEC-2004

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** harmful  
**R-Phrases:** (65) Harmful: may cause lung damage is swallowed

**Source:** German Rapporteur  
21-DEC-2004

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** highly flammable  
**R-Phrases:** (11) Highly flammable

**Source:** German Rapporteur  
21-DEC-2004

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** irritating  
**R-Phrases:** (36/38) Irritating to eyes and skin

**Source:** German Rapporteur  
21-DEC-2004

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** mutagenic, category 2  
**R-Phrases:** (46) May cause heritable genetic damage

**Source:** German Rapporteur  
21-DEC-2004

**Classified:** as in Directive 67/548/EEC  
**Class of danger:** toxic  
**R-Phrases:** (48/23/24/25) Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed

**Source:** German Rapporteur  
21-DEC-2004

### **1.8.1 Occupational Exposure Limit Values**

**Type of limit:** MAC (NL)  
**Limit value:** 30 mg/m<sup>3</sup>

**Remark:** 8 hr.

**Source:** DSM Hydrocarbons B.V. Sittard  
13-DEC-1996

**Type of limit:** MAC (NL)  
**Limit value:** 10 ml/m3

**Remark:** Proposal : 1 ppm  
With the annotation "H" (huid=skin)

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
13-DEC-1996 (282)

**Type of limit:** MAC (NL)  
**Limit value:** 30 mg/m3

**Remark:** Skin notation  
Listed as carcinogen

**Source:** Shell Nederland Chemie B.V. Rotterdam (Pernis)  
03-AUG-2000 (281)

**Type of limit:** MAK (DE)  
**Limit value:** 2.5 ml/m3

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassau-  
gerhaus), Tankfeld in der Mineraloelindustrie, Reparatur  
und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
03-AUG-2000 (1114)

**Type of limit:** MAK (DE)  
**Limit value:** 8 mg/m3

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassau-  
gerhaus), Tankfeld in der Mineraloelindustrie, Reparatur  
und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
03-AUG-2000 (1114)

**Type of limit:** MAK (DE)  
**Limit value:** 1 ml/m3

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
03-AUG-2000 (1114)

**Type of limit:** MAK (DE)  
**Limit value:** 3.2 mg/m3

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Remark:** hautresorptiv, krebserzeugend

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4

03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Remark:** krebserzeugend, EG-Kategorie 1

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4

03-AUG-2000 (1117)

**Type of limit:** MAK (DE)

**Country:** Germany

**Remark:** Benzene(71-43-2) is assigned to Category A, and as such has no concentration value listed since no values have been established for a safe concentration range. Special protective and surveillance measures are required.

**Source:** Bitmac Limited Scunthorpe.

13-DEC-1996 (694)

**Type of limit:** MAK (DE)

**Limit value:** 2.5 ml/m<sup>3</sup>

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen

03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Limit value:** 8 mg/m<sup>3</sup>

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen

03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Limit value:** 1 ml/m<sup>3</sup>

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen

03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Limit value:** 3.2 mg/m<sup>3</sup>

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen

03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Remark:** hautresorptiv, krebserzeugend

**Source:** BASF AG Ludwigshafen  
03-AUG-2000 (1114)

**Type of limit:** MAK (DE)

**Remark:** krebserzeugend, EG-Kategorie 1

**Source:** BASF AG Ludwigshafen  
03-AUG-2000 (1117)

**Type of limit:** MAK (DE)  
**Limit value:** mg/m<sup>3</sup>

**Country:** Germany

**Remark:** Benzene is in the carcinogenic group IIIA1, i.e. it is capable of inducing malignant tumors as shown by experience with humans and has a skin notification (H= Danger of cutaneous absorption).

**Source:** Dow Benelux N.V. AA Terneuzen  
13-DEC-1996 (297)

**Type of limit:** MAK (DE)  
**Limit value:** 2.5 ml/m<sup>3</sup>

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen  
18-JAN-1994 (1158)

**Type of limit:** MAK (DE)  
**Limit value:** 8 mg/m<sup>3</sup>

**Remark:** Gilt fuer Kokereien (Dickteerscheider, Kondensation, Gassaugerhaus), Tankfeld in der Mineraloelindustrie, Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen.

**Source:** BASF AG Ludwigshafen  
18-JAN-1994 (1158)

**Type of limit:** MAK (DE)  
**Limit value:** 1 ml/m<sup>3</sup>

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (1158)

**Type of limit:** MAK (DE)  
**Limit value:** 3.2 mg/m<sup>3</sup>

**Remark:** gilt fuer Benzol im uebrigen

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (1158)

**Type of limit:** MAK (DE)

**Remark:** hautresorptiv, krebserzeugend

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (1158)

**Type of limit:** MAK (DE)

**Remark:** krebserzeugend, EG-Kategorie 1

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (1157)

**Type of limit:** MEL (UK)  
**Limit value:** 16 mg/m3

**Source:** Bitmac Limited Scunthorpe.  
13-DEC-1996 (494)

**Type of limit:** MEL (UK)  
**Limit value:** 30 mg/m3

**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
13-DEC-1996

**Type of limit:** MEL (UK)  
**Limit value:** 5 ml/m3

**Source:** ICI Chemicals & Polymers Limited Runcorn, Cheshire  
13-DEC-1996

**Type of limit:** MEL (UK)  
**Limit value:** 5 ml/m3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
13-DEC-1996 (1180)

**Type of limit:** MEL (UK)  
**Limit value:** 16 mg/m3

**Country:** United Kingdom

**Remark:** Benzene is defined as carcinogen for the purpose of the COSHH Regulation as at 1 January 1993 and to which the special provisions for carcinogen in the COSHH Regulations apply.

**Source:** Dow Benelux N.V. AA Terneuzen  
13-DEC-1996 (322)

**Type of limit:** MEL (UK)  
**Limit value:** 16 mg/m3

**Country:** United Kingdom

**Source:** Shell UK Ltd. London  
03-AUG-2000 (493)

## 1. General Information

Substance ID: 71-43-2

**Type of limit:** MEL (UK)  
**Limit value:** 16 mg/m3

**Source:** Rhone-Poulenc Chemicals LTD WATFORD, HERTFORSHIRE  
 13-DEC-1996

**Type of limit:** MEL (UK)  
**Limit value:** 16 mg/m3

**Country:** United Kingdom  
**Source:** Shell UK Limited, London  
 03-AUG-2000 (493)

**Type of limit:** OES (UK)  
**Limit value:** 2 mg/m3

**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (532)

**Type of limit:** OES (UK)  
**Limit value:** 2 mg/m3

**Remark:** Reference not yet available (12/10/94)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997 (532)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** confirmed human carcinogen  
**Source:** BASF AG Ludwigshafen  
 BASF Antwerpen N. V. Antwerpen 4  
 13-DEC-1996 (6)

**Type of limit:** TLV (US)

**Remark:** limit value: 10 ppm. Confirmed human carcinogen.  
**Source:** BASF AG Ludwigshafen  
 BASF Antwerpen N. V. Antwerpen 4  
 13-DEC-1996 (6)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** A 1992-1993 Notice of Intended Changes has also been published by US ACGIH to lower the benzene TWA to 0.1 ppm (0.3 mg/m3) with a skin notation and A1 (confirmed human carcinogen).  
 A2 (suspected human carcinogen) notation given.

**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (10)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Country:** United States of America  
**Remark:** Benzene(71-43-2) is assigned to Appendix A2- a Suspected Human Carcinogen. The TLV List of 1993-1994 includes Benzene as being under Notice of Intended Change with 0.3 mg/m3 as the value and reassignment to Appendix A1- a Known Human Carcinogen with an additional Skin notation.  
**Source:** Bitmac Limited Scunthorpe.  
13-DEC-1996 (2)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** confirmed human carcinogen  
**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (6)

**Type of limit:** TLV (US)  
**Remark:** limit value: 10 ppm. Confirmed human carcinogen.  
**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (6)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
13-DEC-1996 (8)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** TLV-TWA: 32, A2 mg/m3  
Intended change:  
TLV-TWA: 0.3, A1 mg/m3  
Notes:skin

**Source:** A1= Confirmed Human Carcinogens  
A2= Suspected Human Carcinogens  
ENICHEM AUGUSTA INDUSTRIALE Srl Milan  
13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** TLV-TWA = 10 ppm = 32 mg/m3  
**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** A2: Suspected Human Carcinogen: The agent is carcinogenic in experimental animals at dose levels, by route(s) of administration, at site(s), of histologic type(s), or by mechanism(s) that are considered relevant to worker exposure. Available epidemiologic studies are conflicting or insufficient to confirm an increased risk of cancer in exposed humans.

**Source:** Enichem S.p.A. Milan  
 03-AUG-2000

(7)

**Type of limit:** TLV (US)  
**Limit value:** .3 mg/m3

**Remark:** A1: Confirmed Human Carcinogen: The agent is carcinogenic to humans based on the weight of evidence from epidemiologic studies of, or convincing clinical evidence in, exposed humans.

Notice of Intended Changes (1993-1994)  
**Source:** Enichem S.p.A. Milan  
 03-AUG-2000

(7)

**Type of limit:** TLV (US)  
**Limit value:** 10 ml/m3

**Remark:** Benzene has been indicated as category A2 carcinogen: "Suspected Human Carcinogen."

The USA ACGIH has proposed a TLV for Benzene of 0.3 ppm.  
**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt

13-DEC-1996

(1130)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Country:** USA

**Remark:** For the exposure value and the classification as A2 carcinogen ('suspected human carcinogen') following changes are intended: TWA = 0.3 mg/m3, A1 carcinogen ('confirmed human carcinogen')

**Source:** Dow Benelux N.V. AA Terneuzen  
 13-DEC-1996

(7)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Source:** Elf Atochem Paris La Defense 10  
 13-DEC-1996

(562)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Source:** REPSOL PETROLEO, S.A. MADRID  
 13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** A2 (suspected human carcinogen). A 1992-1993 ACGIH Notice of Intended Changes to lower the benzene TWA to 0.1ppm with a skin notation and A1 (confirmed human carcinogen)

**Source:** PETROLEOS DE PORTUGAL - PETROGAL, S.A. LISBOA  
 13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 10 other: ppm

**Source:** PRAOIL S.R.L. ASSAGO MI  
 13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** PROTEZIONE PERSONALE (IN DIPENDENZA DEL TIPO ED ENTITA' DELLA POSSIBILE ESPOSIZIONE):  
 MASCHERA CON FILTRO PER VAPORI ORGANICI  
 AUTORESPIRATORE  
 GUANTI IN PVC A MANICA LUNGA  
 OCCHIALI A TENUTA  
 SCHERMO FACCIALE

**Source:** INDUMENTI E STIVALI IMPERMEABILI AL BENZENE  
 POLIOLI S.P.A. Cologno Monzese (MI)  
 13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** Listed as suspected human carcinogen  
**Source:** Shell Nederland Chemie B.V. Rotterdam (Pernis)  
 13-DEC-1996

(9)

**Type of limit:** TLV (US)  
**Limit value:** .3 mg/m3

**Source:** TOTAL PARIS LA DEFENSE  
 13-DEC-1996

**Type of limit:** TLV (US)  
**Limit value:** 1 mg/m3

**Remark:** = 0.3 ppm; 8h TWA  
 SK (skin) and A1 (confirmed human carcinogen) notation proposed

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997

(11)

**Type of limit:** TLV (US)  
**Limit value:** 1.6 mg/m3

**Source:** Compañia Española de Petroleos CEPSA Madrid  
 20-NOV-1998

**Type of limit:** TLV (US)  
**Limit value:** .5 other: ppm

**Short term exposure**

**Limit value:** 2.5 other: ppm  
**Schedule:** 15 minute(s)  
**Frequency:** 4 times

**Source:** ACGIH, 2002  
ENI SpA - Divisione Refining & Marketing ROMA  
30-MAY-2003

**Type of limit:** TLV (US)  
**Limit value:** 1.6 mg/m3

**Short term exposure**

**Limit value:** 8 mg/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 4 times

**Remark:** LIMITI ACGIH:  
TLV/TWA: 0,5 ppm A1  
1,6 mg/m3 A1  
TLV/STEL: 2,5 ppm A1  
8 mg/m3 A1  
(Skin)

**Source:** ACNA C.O. in Liquidazione Cengio (Savona)  
29-OCT-1998 (3)

**Type of limit:** TLV (US)  
**Limit value:** 32 mg/m3

**Remark:** confirmed human carcinogen

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (6)

**Type of limit:** TLV (US)

**Remark:** limit value: 10 ppm. Confirmed human carcinogen.

**Source:** BASF AG Ludwigshafen  
06-JAN-1994 (6)

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m3

**Remark:** Wert für : übrige (Anwendungen)  
**Source:** BRENNTAG AG Mülheim an der Ruhr  
13-DEC-1996

**Type of limit:** TRK (DE)

**Remark:** TRGS 900 (1993): Technische Richtkonzentrationen (TRK-Wert)  
 - Kokereien (Dickteerschneider, Kondensation, Gassaugerhaus)  
 = 2,5 ppm / 8 mg/m<sup>3</sup>  
 - Tankfeld in der Mineralölindustrie = 2,5 ppm / 8 mg/m<sup>3</sup>  
 - Reparatur und Wartung von Ottokraftstoff bzw. benzol-  
 führenden Teilen = 2,5 / 8 mg/m<sup>3</sup>  
 - im übrigen = 1,0 ppm / 3,2 mg/m<sup>3</sup>

**Source:** Aral Aktiengesellschaft Bochum  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m<sup>3</sup>

**Remark:** bei Tankfeld in der Mineralölindustrie  
**Source:** DEA Mineraloel AG Hamburg  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m<sup>3</sup>

**Remark:** im übrigen  
**Source:** DEA Mineraloel AG Hamburg  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m<sup>3</sup>  
**Short term exposure**  
**Limit value:** 12.5 ml/m<sup>3</sup>  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** TRK value valid for coke refineries, area of storage tanks  
 in the mineral oil industry and repair and maintenance of  
 fuel or benzene containing parts.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 13-DEC-1996

(1115)

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m<sup>3</sup>  
**Short term exposure**  
**Limit value:** 5 ml/m<sup>3</sup>  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** Valid for all other applications except those mentioned  
 above.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 03-AUG-2000

(1115)

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3

**Remark:**  
 - für Kokereien  
 - Tankfeld in der Mineralölindustrie  
 - Reparatur und Wartung von Ottokraftstoff bzw. Benzol führenden Teilen

**Source:** EC Erdölchemie GmbH Köln (295)  
 03-AUG-2000

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m3

**Remark:** - im übrigen (alle weiteren Anwendungen)

**Source:** EC Erdölchemie GmbH Köln (295)  
 03-AUG-2000

**Type of limit:** TRK (DE)  
**Limit value:** 16 mg/m3

**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m3

**Remark:** With derogations  
**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 8 mg/m3

**Country:** Germany  
**Remark:** Benzene (Benzol)  
 =====

Verwendung	TRK
	(Technische Richtkonzentration)

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Kokereien (Dickteerschneider, Kondensation, Gassaugerhaus)	8 mg/m3

Tankfeld in der Mineraloelindustrie	8 mg/m3
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Reparatur und Wartung von Ottokraftstoff bzw. benzolfuehrenden Teilen	8 mg/m3
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im uebrigen	3.2 mg/m3
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**Source:** Dow Benelux N.V. AA Terneuzen (100)  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 16 mg/m3

**Source:** Elf Atochem Paris La Defense 10  
13-DEC-1996 (562)

**Type of limit:** TRK (DE)  
**Limit value:** 3.2 mg/m3

**Source:** Ruetgerswerke AG Duisburg  
03-AUG-2000 (295)

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3

**Short term exposure**

**Limit value:** 12.5 ml/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** -für Kokereien (Dickteerscheider, Kondensation,  
Gassaugerhaus)

**Source:** ÖMV Deutschland GmbH Burghausen  
13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3

**Short term exposure**

**Limit value:** 12.5 ml/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** -Tankfeld in der Mineralölindustrie

**Source:** ÖMV Deutschland GmbH Burghausen  
13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3

**Short term exposure**

**Limit value:** 12.5 ml/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** -Reparatur und Wartung von  
Ottokraftstoff-bzw. benzenführenden Teilen

**Source:** ÖMV Deutschland GmbH Burghausen  
13-DEC-1996

**Type of limit:** TRK (DE)**Limit value:** 1 ml/m3**Short term exposure****Limit value:** 5 ml/m3**Schedule:** 15 minute(s)**Frequency:** 5 times**Remark:** -im übrigen**Source:** ÖMV Deutschland GmbH Burghausen

03-AUG-2000

(695) (1116)

**Type of limit:** TRK (DE)**Limit value:** 3.2 mg/m3**Source:** Ruetgers-VfT AG Duisburg

03-AUG-2000

(295)

**Type of limit:** TRK (DE)**Limit value:** 1 ml/m3**Short term exposure****Limit value:** 5 ml/m3**Schedule:** 15 minute(s)**Frequency:** 5 times**Source:** PCK AG Schwedt Schwedt/Oder

13-DEC-1996

**Type of limit:** TRK (DE)**Limit value:** 3.2 mg/m3**Short term exposure****Limit value:** 16 mg/m3**Schedule:** 15 minute(s)**Frequency:** 5 times**Remark:** Zum Kurzzeitwert: Der zeitliche Abstand von einer Expositionsspitze zur anderen darf 1h nicht unterschreiten.**Source:** PCK AG Schwedt Schwedt/Oder

13-DEC-1996

(1156) (1159)

**Type of limit:** TRK (DE)**Limit value:** 16 mg/m3**Source:** ÖMV - Chemie Linz GMBH Linz

13-DEC-1996

**Type of limit:** TRK (DE)**Limit value:** 2.5 ml/m3**Source:** Ruhr Oel GmbH Gelsenkirchen-Hassel

13-DEC-1996

**Type of limit:** TRK (DE)**Limit value:** 8 mg/m3**Source:** Sächsische Olefinwerke GmbH Böhlen

03-AUG-2000

(1114)

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3

**Source:** Sächsische Olefinwerke GmbH Böhlen  
 13-DEC-1996

**Type of limit:** TRK (DE)  
**Limit value:** 16 mg/m3

**Country:** Federal Republic of Germany  
**Remark:** group III A1 (confirmed human carcinogen)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997 (298)

**Type of limit:** TRK (DE)  
**Limit value:** 2.5 ml/m3  
**Short term exposure**  
**Limit value:** 12.5 ml/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** TRK value valid for coke refineries, area of storage tanks  
 in the mineral oil industry and repair and maintenance of  
 fuel or benzene containing parts.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 25-MAY-1994 (1115)

**Type of limit:** TRK (DE)  
**Limit value:** 1 ml/m3  
**Short term exposure**  
**Limit value:** 5 ml/m3  
**Schedule:** 15 minute(s)  
**Frequency:** 5 times

**Remark:** Valid for all other applications except those mentioned  
 above.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 25-MAY-1994 (1113)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** France  
**Remark:** Type of Limit: VME  
**Source:** Atochem Paris la Defense  
 13-DEC-1996 (561)

**Type of limit:** other  
**Limit value:** 32 mg/m3

**Country:** Belgium  
**Remark:** C2 (suspected human carcinogen) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

## 1. General Information

Substance ID: 71-43-2

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** Denmark, Finland, Switzerland  
**Remark:** SK (skin) and C or, for Finland, C3 (suspected carcinogen) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** France  
**Remark:** C (suspected carcinogen) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

**Type of limit:** other  
**Limit value:** 3 mg/m3

**Country:** Sweden  
**Remark:** SK (skin) and C3 (suspected carcinogenic potential) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

**Type of limit:** other  
**Limit value:** 32 mg/m3

**Country:** Former USSR  
**Remark:** SK (skin) and C (suspected carcinogen) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** Australia  
**Remark:** Carcinogen category 1 (confirmed human carcinogen).  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (835)

**Type of limit:** other  
**Short term exposure**  
**Limit value:** 30 mg/m3

**Country:** Finland  
**Remark:** SK (skin) and C3 (suspected carcinogenic potential) notation given.  
**Source:** BP Chemicals Ltd LONDON  
 13-DEC-1996 (557)

**Type of limit:** other**Short term exposure****Limit value:** 16 mg/m3**Country:** Sweden**Remark:** SK (skin) and C3 (suspected carcinogenic potential) notation given.**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

(557)

**Type of limit:** other**Short term exposure****Limit value:** 80 mg/m3**Country:** Former USSR**Remark:** SK (skin) and C (suspected carcinogen) notation given.**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

(557)

**Type of limit:** other**Limit value:** 15 mg/m3**Remark:** MAC (ex URSS), 1980-1987  
Value for vapour (for gas 5 mg/m3).**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
13-DEC-1996**Type of limit:** other**Limit value:** 16 mg/m3**Country:** France**Remark:** Type of Limit: VME**Source:** Elf Atochem Paris La Defense 10  
13-DEC-1996

(561)

**Type of limit:** other**Country:** United Kingdom**Remark:** Internal Working Limit**Source:** 3 mg/m3 8 hour TWA  
Shell UK Ltd. London**Test substance:** Benzene  
13-DEC-1996**Type of limit:** other**Country:** United Kingdom**Remark:** Internal Working Limit**Source:** 3 mg/m3 8 hour TWA  
Shell UK Limited, London**Test substance:** Benzene  
13-DEC-1996

**Type of limit:** other  
**Limit value:** 30 mg/m3

**Remark:** NP 1796 (1988) Portuguese Standard  
**Source:** PETROLEOS DE PORTUGAL - PETROGAL, S.A. LISBOA  
 13-DEC-1996

**Type of limit:** other  
**Limit value:** 32 mg/m3

**Source:** TOTAL PARIS LA DEFENSE  
 13-DEC-1996

**Type of limit:** other  
**Short term exposure**  
**Limit value:** 80 mg/m3

**Source:** TOTAL PARIS LA DEFENSE  
 13-DEC-1996

**Type of limit:** other  
**Short term exposure**  
**Limit value:** 16 mg/m3

**Source:** TOTAL PARIS LA DEFENSE  
 13-DEC-1996

**Type of limit:** other  
**Limit value:** 32 mg/m3

**Country:** Belgium  
**Remark:** C2 (suspected human carcinogen) notation given.  
 Reference not yet available (12/10/94)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997 (557)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** Denmark, Finland, Switzerland  
**Remark:** Reference not yet available (12/10/94)  
 SK (skin) and C or, for Finland, C3 (suspected carcinogen)  
 notation given.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997 (557)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** France  
**Remark:** C (suspected carcinogen) notation given.  
 Reference not yet available (12/10/94)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 06-JAN-1997 (557)

**Type of limit:** other  
**Limit value:** 3 mg/m3

**Country:** Sweden  
**Remark:** Reference not yet available (12/10/94)  
SK (skin) and C3 (suspected carcinogenic potential) notation given.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (557)

**Type of limit:** other  
**Limit value:** 32 mg/m3

**Country:** Former USSR  
**Remark:** Reference not yet available (12/10/94)  
SK (skin) and C (suspected carcinogen) notation given.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (557)

**Type of limit:** other  
**Limit value:** 16 mg/m3

**Country:** Australia  
**Remark:** Carcinogen category 1 (confirmed human carcinogen).  
Reference not yet available (12/10/94)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (835)

**Type of limit:** other  
**Short term exposure**  
**Limit value:** 30 mg/m3

**Country:** Finland  
**Remark:** Reference not yet available (12/10/94)  
SK (skin) and C3 (suspected carcinogenic potential) notation given.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (557)

**Type of limit:** other  
**Short term exposure**  
**Limit value:** 16 mg/m3

**Country:** Sweden  
**Remark:** Reference not yet available (12/10/94)  
SK (skin) and C3 (suspected carcinogenic potential) notation given.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (557)

**Type of limit:** other

**Short term exposure**

**Limit value:** 80 mg/m3

**Country:** Former USSR

**Remark:** Reference not yet available (12/10/94)  
SK (skin) and C (suspected carcinogen) notation given.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(557)

**Type of limit:** other

**Limit value:** 3.2 mg/m3

**Country:** Belgium

**Remark:** 1ppm  
skin annotation

**Source:** Bayer Antwerpen

21-OCT-2004

**Type of limit:** other

**Remark:** Occupational exposure limits (2004):

USA

U.S. Federal, Workplace

ACGIH Threshold Limit Values (2004)

The 8-Hour Exposure Limit (TLV-TWA) is 0.5 ppm.

The 15-minute STEL is 2.5 ppm.

OSHA Specifically Regulated Substances (29 CFR 910.1001-.1052)

OSHA TWA is 1 ppm.

OSHA STEL is 5 ppm.

OSHA Table Z-2 (29 CFR 1910.1000)

The 8-hour TWA is 10 ppm.

The Ceiling Limit is 25 ppm.

The acceptable max. peak above the ceiling concentration for an 8-hour shift is 50 ppm.

The acceptable duration of the peak above the ceiling concentration is 10 minutes

EU - OELs from Annex III, Part A (Limit values for occupational exposure) to Directive 2004/37/EC (OJ (L 229) 23, 29 June 2004)

The 8-hour Limit Value is 1 ppm ; 3,25 mg/m3.

Austria - TRK List (Annex II and III). Grenzwerteverordnung

2003 as amended by BGB1. II, number 119, 10 March 2004

The TRK TWA is 1 ml/m3 (ppm) ; 3,2 mg/m3.

The TRK STEL is 4 ml/m3 (ppm) ; 12,8 mg/m3.

Belgium - Exposure Limit Values. 2002 Moniteur Belge number

341, 25 October 2002

The 8-hour TWA is 1 ppm ; 3,25 mg/m3.

Denmark - OELs. National Labour Inspectorate. Exposure Limit Values for Substances and Materials (Arbejdstilsynet).

Grønseværdier for stoffer og materialer), October 2002  
TWA is 0,5 ppm ; 1,6 mg/m<sup>3</sup>.

Finland - Workplace Exposure Limits [HTP-Arvot 2002, Sosiaali- ja Terveysministeriö, Kemian Työsuojeluneuvottelukunta, Tampere 2002]  
Finnish 8-hour limit is 1 ppm ; 3,25 mg/m<sup>3</sup>.

France - OELs (VLR) (INRS, Occupational Exposure Limits To Dangerous Substances in France, December 2003)  
Time Weighted Average (VME) is 1 ppm ; 3,25 mg/m<sup>3</sup>.

Germany - TRGS 900, Limit Values in the Ambient Air (MAK and TRK values) [BArbBl. 10/2000, as amended through 7-8/2004]  
TRGS 900 limit value is 1 ml/m<sup>3</sup> (ppm) ; 3,25 mg/m<sup>3</sup>.  
TRGS 900 15-minute limit (see notes): 4  
Note: 4 The average concentrations of substances with TRK values, shall never exceed 4 times the MAK limit value (15 minute average - MAK Excess Factor 4). The 8-hour time weighted average must always be observed.

Ireland - OELs (2002 Code of Practice for the Safety, Health and Welfare at Work [Chemical Agents] Regulations)  
Irish 8-hour OEL (TWA) is 1 ppm ; 3 mg/m<sup>3</sup>.

Italy - OELs (as amended 26 February 2004)  
The 8-hour TWA is 1 ppm ; 3,25 mg/m<sup>3</sup>.

Luxembourg - OELs for Carcinogens/Mutagens (26 August 2002)  
The 8-hour Limit Value is 1 ppm ; 3.25 mg/m<sup>3</sup>.

The Netherlands - National MAC List 2004  
MAC TWA (TGG): 1 ppm ; 3,25 mg/m<sup>3</sup>.

Norway - Administrative Norms for Contaminants in the Workplace 2003 [Administrative normer for forurensning i arbeidsatmosfære 2003] No. 361  
The threshold limit value is 1 ppm ; 3 mg/m<sup>3</sup>.

Spain - Carcinogens and Mutagens with Limit Values (OEL list, 2004)  
The 8-hour daily exposure limit (VLA-ED) is 1 ppm ; 3,25 mg/m<sup>3</sup>.

Sweden - OELs. National Board of Occupational Safety and Health, Occupational Exposure Limit Values (AFS 2000)  
Level Limit Value (NGV) 0,5 ppm ; 1,5 mg/m<sup>3</sup>.  
Short Term Limit (KTV) 3 ppm ; 9 mg/m<sup>3</sup>.

Switzerland - SUVA. Grenzwerte am Arbeitsplatz 2003 [Limit Values at the Workplace 2003]  
TWA is 1 ppm ; 3,2 mg/m<sup>3</sup>.

United Kingdom - UK. Health and Safety Executive. EH40/2002; Occupational Exposure Limits 2002 (including Supplement 2003)  
TWA is 1 ppm.

**Source:** German rappateur

13-JAN-2005

**Type of limit:** other: Ireland  
**Limit value:** 5 ml/m3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

**Type of limit:** other: Italy  
**Limit value:** 10 ml/m3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

**Type of limit:** other: Norway  
**Limit value:** 1 ml/m3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

**Type of limit:** other: PEL(OSHA): Permitted Exposure Limit  
**Limit value:** 1 ml/m3

**Short term exposure**  
**Limit value:** 5 ml/m3

**Source:** REPSOL PETROLEO, S.A. MADRID  
 13-DEC-1996

(860)

**Type of limit:** other: Sweden  
**Limit value:** .5 ml/m3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

**Type of limit:** other: TLV  
**Limit value:** 3 other: ppm

**Source:** Direttiva 97/42/CE  
 ENI SpA - Divisione Refining & Marketing ROMA  
 09-JUL-1999

**Type of limit:** other: TLV (Spanish Ministry of Labour)  
**Short term exposure**  
**Limit value:** 25 ml/m3

**Source:** REPSOL PETROLEO, S.A. MADRID  
 13-DEC-1996

**Type of limit:** other: TRGS 900

**Remark:** TRK-Wert: 1 ppm = 3,25 mg/m3 Spitzenbegrenzung Kategorie: 4  
 Gefahr der Hautresorption

**Source:** Bayer MaterialScience  
 01-JUL-2004

**Type of limit:** other: TWA (Belgium)  
**Limit value:** 10 ml/m3

**Remark:** Benzene has been indicated as a C2 "suspected human carcinogen".

Proposal : 3 ppm

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

(851)

**Type of limit:** other: VME  
**Limit value:** 16 mg/m3

**Short term exposure**

**Limit value:** 3 mg/m3

**Remark:** Use local exhaust ventilation.  
 Hand protection : nitrile rubber gloves.  
 Eye protection : safety monogoggles.  
 Body protection : standard issue work clothes.  
 chemicals resistant shoes or boots.  
 if splashes are likely to occur,  
 wear : PVC apron.

**Source:** SHELL FRANCE Rueil Malmaison  
 13-DEC-1996

**Type of limit:** other: VME  
**Limit value:** 16 mg/m3

**Short term exposure**

**Limit value:** 3 mg/m3

**Remark:** Use local exhaust ventilation.  
 Hand protection : nitrile rubber gloves.  
 Eye protection : safety monogoggles.  
 Body protection : standard issue work clothes.  
 chemicals resistant shoes or boots.  
 if splashes are likely to occur,  
 wear : PVC apron.

**Source:** SHELL CHIMIE RUEIL MALMAISON  
 13-DEC-1996

**Type of limit:** other: VME (France)  
**Limit value:** 5 ml/m3

**Remark:** VME = Valeur Moyenne d'Exposition

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

(211)

**Remark:** Gefahr der Hautresorption

**Source:** EC Erdölchemie GmbH Köln  
 03-AUG-2000

(295)

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
 13-DEC-1996

(1280)

### 1.8.3 Water Pollution

**Classified by:** KBwS (DE)  
**Labelled by:** KBwS (DE)  
**Class of danger:** 3 (strongly water polluting)

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
01-JUL-2004

**Classified by:** KBwS (DE)  
**Class of danger:** 3 (strongly water polluting)

**Source:** Bayer MaterialScience  
01-JUL-2004

### 1.8.4 Major Accident Hazards

**Legislation:** Stoerfallverordnung (DE)  
**Substance listed:** yes

**Remark:** Stoerfall-Stoff-Nr. 39  
**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
13-DEC-1996 (1091)

**Legislation:** Stoerfallverordnung (DE)  
**Substance listed:** yes

**Remark:** Anhang II, Nr. 39  
**Source:** Bayer MaterialScience  
01-JUL-2004

### 1.8.5 Air Pollution

**Classified by:** TA-Luft (DE)  
**Labelled by:** TA-Luft (DE)  
**Number:** 2.3 (carcinogenic substances)  
**Class of danger:** III

**Source:** BASF AG Ludwigshafen  
BASF Antwerpen N. V. Antwerpen 4  
13-DEC-1996

**Classified by:** TA-Luft (DE)  
**Number:** 2.3 (carcinogenic substances)  
**Class of danger:** III

**Source:** Bayer MaterialScience  
01-JUL-2004

### 1.10 Source of Exposure

**Remark:**

In the framework of the second German Environmental Survey carried out in the Western part of Germany in 1990/91 (GerES IIa) 113 adults aged 25-69 years were selected at random from the total study population of about 2500 to investigate personal exposure to about 70 volatile organic compounds (VOCs). Each subject wore a diffusive badge-type sampler for 1 week. The VOCs determined included alkanes, aromatics, aliphatic halocarbons, terpenes, and oxygen-containing compounds. Multivariate regression analysis was carried out to determine and quantify the major sources of personal exposure to various VOCs. In this paper, results are given for benzene, and C8- and C9-aromatics. Being subject to environmental tobacco smoke was found to be the most important determinant of benzene exposure, but automobile-related activities such as driving a car or refuelling, were also associated with significantly increased levels of benzene. The major determinant of C8- and C9-aromatics concentrations was occupational exposure. Emissions from paints, lacquers, newspapers, magazines and print-works were also important contributors to C8-aromatics exposure. Renovation, painting and smoking were associated with a significant increase of the exposure to C9-aromatics.

Additional Comments: In a population sample of 113 subjects, the 95% confidence interval for the geometric mean concentration of benzene was found to be between 9.3 and 11.9 microgram/m<sup>3</sup> during the one week monitoring/sampling period. The results of such personal monitoring, in combination with information on time activity patterns, can be used to establish population exposure distributions for VOCs if a large enough number of individuals is studied. Multivariate regression analysis was applied successfully to determine major sources of personal exposure to a number of aromatic VOCs. For benzene, 40% of the total variance could be explained with exposure to environmental tobacco smoke and automobile-related activities having the highest influence on exposure.

The German Environmental Survey 1990/92 (GerES II): sources of personal exposure to volatile organic compounds.

**Source:****Reliability:**

20-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(514)

**Remark:**

Benzene exposures caused by traffic in Munich public transportation systems between 1993 and 1997. Volatile aromatics (benzene, toluene, xylene, the BTX-aromatics.) were measured from 1993 to 1997 in buses and trams in the center of Munich, Germany, and along main roads during regular rides. Sampling time was between 07.00 and 00.00 h. A total of 631 probes were sampled and centrally analyzed. In the 5-yr mean, 15.0 microgram benzene/m<sup>3</sup>, 50% above the limit of the 23 BImSchV and 107.5 microgram BTX

aromates/m<sup>3</sup> along strongly traffic-loaded main streets, were observed. Splitting up these mean emissions into single years, a trend toward a decline of mean emissions of all volatile aromatics (benzene from 23.8 to 7.4 micrograms/m<sup>3</sup>) and the sum of BTX aromatics (from 147.5 to 59.4 microgram/m<sup>3</sup>) was observed. Measured hydrocarbon concentrations in Munich center were consistent with long-range theoretical calculations concerning the decrease of traffic-caused benzene emissions in cities. If current trends continue, benzene concentrations are expected to be below 5 microgram/m<sup>3</sup> by the year 2001 and below 2.5 microgram/m<sup>3</sup> by the year 2008. At these levels, the carcinogenic risk from benzene is probably less significant than public health risks from other car exhaust components.

**Source:**  
**Reliability:**  
24-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(955)

**Remark:**

Commuter exposure to volatile organic compounds under different driving conditions. The driving conditions that were tested for the in-vehicle concentrations of selected volatile organic compounds (VOCs) included transport modes, fuel distributions, vehicle ventilation conditions, driving routes, commute seasons, car models, and driving periods. This study involved two sampling seasons (winter and summer). The in-auto/in-bus/fixed site ratio of the wintertime mean concentrations was about 6/3/1 for total VOCs and 8/3/1 for benzene. On the median, the in-auto/in-bus exposure ratio ranged from 1.5 to 2.8 for the morning commutes, and ranged from 2.4 to 4.5 for evening commutes, depending on the target compounds. The wintertime in-auto concentrations were significantly higher ( $p < 0.05$ ), on the average 3-5 times higher, in a carbureted engine than in the three electronic fuel-injected cars. For the summertime in-auto concentrations of the target compounds except benzene, there were no significant differences between low and high ventilation conditions on the two urban routes. The urban in-auto benzene concentration was significantly higher ( $p < 0.05$ ) under the low ventilation condition. For the rural commutes, the in-auto concentrations of all target compounds were significantly higher ( $p < 0.05$ ) under the low ventilation condition. The in-auto VOC concentrations on the two urban routes did not differ significantly, and they were greater than the rural in-auto concentrations, with the differences being significant ( $p < 0.05$ ) for all target compounds. The summertime in-auto concentrations of benzene and toluene were greater than the wintertime in-auto concentrations, with the difference being significant ( $p < 0.05$ ), while the concentrations of the other target compounds were not significantly different between the two seasons. Neither car models nor driving periods influenced the in-auto VOC concentrations.

**Source:**  
**Reliability:**  
24-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(583)

**Remark:** Airborne concentrations of benzene, toluene and the xylenes have been measured inside passenger cars whilst driven along major roads in the city of Birmingham, UK, as well as immediately outside the car, and at the roadside. A comparison of concentrations measured in the car with those determined from immediately outside showed little difference, with a mean ratio for benzene of  $1.17 \pm 0.34$  and for toluene  $1.11 \pm 0.16$  (n=53). The ratio of in-car to roadside concentration was rather higher at  $1.55 \pm 0.68$  for benzene and  $1.54 \pm 0.72$  for toluene (n=53). The roadside concentrations were typically several-fold higher than those measured at a background suburban monitoring station within Birmingham, although much variation was seen between congested and uncongested roads, with concentrations adjacent to uncongested roads similar to those measured at the background monitoring station. Measurements of benzene and toluene in a car driven on a rural road outside the city showed very comparable in-car and out-of-car concentrations strengthening the conclusion that pollution inside the car is derived from pollutants outside entering with ventilation air. The exceptions were an older car where in-car concentrations appreciably exceeded those outside (in-to out-vehicle ratio=2.3 for benzene and 2.2 for toluene where n=5) indicating probable self-contamination, and a very new car which built up increased VOC concentrations when stationary without ventilation (in-to out-vehicle ratio=2.4 for benzene and 3.3 for toluene where n=5). A further set of measurements inside London taxi cabs showed concentrations to be influenced by the area within which the taxi was driven, the traffic density and the presence of passengers smoking cigarettes. Roadside and in-vehicle concentrations of monoaromatic hydrocarbons.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
24-JUL-2000 (682)

**Remark:** Atmospheric carcinogens in Rio de Janeiro during the summer of 1998/99: benzo[a]pyrene and benzene. Rio de Janeiro, the second largest city in Brazil, is affected by severe pollution episodes and presents a high respiratory cancer incidence in comparison with the rest of the country. To monitor atmospheric pollution during the summer of 1998/1999 and to estimate the impact of organic pollution on public health, we determined the levels of two carcinogenic organic chemicals, benzo[a]pyrene and benzene, in four distinct sites throughout the city. A review of the levels recorded in other urban areas worldwide during the last ten years indicates that the benzo[a]pyrene (< or = 0.70 ng/m<sup>3</sup>) and benzene (< or = 11 micrograms/m<sup>3</sup>) concentrations found in Rio are relatively low. The highest levels were generally recorded in developing Asian countries, whereas the lowest values were found in North America. Unlike urban areas in temperate zones, pollution derived from domestic heating is minor in Rio de Janeiro,

where most of the benzo[a]pyrene and benzene pollution originates from vehicular traffic. The quite distinct fuels used in light-duty vehicles in Brazil, combined with strong light incidence and increased rainfall during the summer, also contribute to diminish the levels of such pollutants.

Additional Comments: In this study, benzene in the air in Rio varied from nondetectable to 11 microgram/m<sup>3</sup> and only 2 out of 12 samples presented concentrations > 6.5 microgram/m<sup>3</sup> (2 ppb). Ambient air standards for benzene in Brazil were established at about 10 microgram/m<sup>3</sup> (3 ppb). EXXON Biomedical Sciences East Millstone, NJ

**Source:**  
**Reliability:**  
21-JUL-2000

(1) valid without restriction

(353)

**Remark:**

Exposures to jet fuel and benzene during aircraft fuel tank repair in the U.S. Air Force  
Jet fuel and benzene vapor exposures were measured during aircraft fuel tank entry and repair at twelve U.S. Air Force bases. Breathing zone samples were collected on the fuel workers who performed the repair. In addition, instantaneous samples were taken at various points during the procedures with SUMMA canisters and subsequent analysis by mass spectrometry. The highest eight-hour time-weighted average (TWA) fuel exposure found was 1304 mg/m<sup>3</sup>; the highest 15-minute short-term exposure was 10,295 mg/m<sup>3</sup>. The results indicate workers who repair fuel tanks containing explosion suppression foam have a significantly higher exposure to jet fuel as compared to workers who repair tanks without foam (p < 0.001). It is assumed these elevations result from the tendency for fuel, absorbed by the foam, to volatilize during the foam removal process. Fuel tanks that allow flow-through ventilation during repair resulted in lower exposures compared to those tanks that have only one access port and, as a result, cannot be ventilated efficiently. The instantaneous sampling results confirm that benzene exposures occur during fuel tank repair; levels up to 49.1 mg/m<sup>3</sup> were found inside the tanks during the repairs. As with jet fuel, these elevated benzene concentrations were more likely to occur in foamed tanks. The high temperatures associated with fuel tank repair, along with the requirement to wear vapor-permeable cotton coveralls for fire reasons, could result in an increase in the benzene body burden of tank entrants.

**Source:**  
**Reliability:**  
20-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(176)

**Remark:**

C5-C8 Non-methane hydrocarbon measurements in Copenhagen: concentrations, sources and emission estimates  
Hourly measurements of 23 non-methane hydrocarbons (NMHC) ranging from C5 to C8 were made in the busy street, Jagtvej, in central Copenhagen, Netherlands during 5 working days in December 1997. The compounds were concentrated in absorbent tubes by active sampling and subsequently analyzed by

thermal desorption and gas chromatography. Parallel measurements of benzene and toluene using a different technique were performed to validate the results. Good agreement was observed ( $r > 0.97$ ). The aromatic compounds were the most abundant species with average concentrations ranging of 10.2 ppbv for toluene to 2.0 ppbv for ethylbenzene. The average benzene concentrations were 3.4 ppbv. The concentrations of the alkanes were 0.2-2.4 ppbv and alkene concentrations were 0.03-0.4 ppbv. All components were highly correlated with primary emitted CO ( $r > 0.88$ ) and benzene ( $r > 0.89$ ) which identifies petrol-fueled motor vehicles as the major source. The emission factors of the hydrocarbons from petrol-fueled vehicles were calculated on the basis of basis of the emission factor known for benzene (0.11 g/km). Highest emission factors were observed for the aromatic compounds (up to 0.41 g/km for toluene), which is in accordance with the literature. The NMHC content of petrol fuel was also measured by GC-MS analysis. An enhancement of benzene and isoprene was observed relative to o-xylene when comparing emission factors with the NMHC composition of the petrol fuel. Benzene generation during combustion and evaporative emissions of isoprene are possible explanations for this observation.

**Source:**  
**Reliability:**  
24-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(202)

**Remark:**

JP-8 jet fuel (similar to commercial/international jet A-1 fuel) is the standard military fuel for all types of vehicles, including the U.S. Air Force aircraft inventory. As such, JP-8 presents the most common chemical exposure in the Air Force, particularly for flight and ground crew personnel during preflight operations and for maintenance personnel performing routine tasks. Personal exposure at an Air Force base occurs through occupational exposure for personnel involved with fuel and aircraft handling and/or through incidental exposure, primarily through inhalation of ambient fuel vapors. Because JP-8 is less volatile than its predecessor fuel (JP-4), contact with liquid fuel on skin and clothing may result in prolonged exposure. The slowly evaporating JP-8 fuel tends to linger on exposed personnel during their interaction with their previously unexposed colleagues. To begin to assess the relative exposures, we made ambient air measurements and used recently developed methods for collecting exhaled breath in special containers. We then analyzed for certain volatile marker compounds for JP-8, as well as for some aromatic hydrocarbons (especially benzene) that are related to long-term health risks. Ambient samples were collected by using compact, battery-operated, personal whole-air samplers that have recently been developed as commercial products; breath samples were collected using our single-breath canister method that uses 1-L canisters fitted with valves and small disposable breathing tubes. We collected breath samples from various groups of Air Force personnel and found a demonstrable JP-8

exposure for all subjects, ranging from slight elevations as compared to a control cohort to > 100 x the control values. This work suggests that further studies should be performed on specific issues to obtain pertinent exposure data. The data can be applied to assessments of health outcomes and to recommendations for changes in the use of personal protective equipment that optimize risk reduction without undue impact on a mission.

Additional Comments: These investigators found that there was an overall moderate benzene exposure at the bases from fuel and exhaust (breath means of 1.7 ppbv vs. controls at 0.60 ppbv), but that smoking causes an additional 400% incremental mean body burden. Personal exposure to JP-8 jet fuel vapors and exhaust at Air Force bases.

**Source:**  
**Reliability:**  
25-JUL-2000

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(898)

**Remark:**

Environmental benzene levels were measured in 26 petrol stations using both active and passive stationary and personal samplers. Simultaneously, benzene levels were measured in the petrol station operators on blood samples collected at the end of the work shift and the following morning before starting work. The petrol stations belonged to various different oil companies and were studied both during the winter (9 stations) and in the summer (17 stations). The environmental levels measured with active samplers in the 26 stations were on average 256 ng/l and were significantly lower (98 ng/l) in winter and higher (326 ng/l) in summer. The blood levels of benzene in 77 workers at the end of the work shift were on average 548 ng/l and were significantly lower (306 ng/l) in winter and higher (651 ng/l) in summer. The following morning, blood levels of benzene were lower than those found at the end of the work shift, on average 249 ng/l in winter and 427 ng/l in summer. Smokers had higher benzene levels than non-smokers, both in winter at the end of the work shift (617/170 ng/l) and the following morning (506/137 ng/l), and in summer at the end of the shift (742/517 ng/l) and the following morning (535/233 ng/l). A comparison with a sample of 243 "normal" subjects of the general population showed that their mean blood level of benzene of 165 ng/l was significantly lower than the level found in petrol station workers the morning after the work shift (364 ng/l).

Environmental exposure and blood benzene levels in gasoline station attendants. Comparison with the general population EXXON Biomedical Sciences East Millstone, NJ

**Source:**  
**Reliability:**  
24-JUL-2000

(2) valid with restrictions

(159)

**Remark:** A survey of personal exposures to benzene in Mexico City. Benzene is a widely distributed environmental contaminant that causes leukemia. It is an important component in gasoline, it is used frequently as a solvent or chemical feedstock in industry, and it is emitted as a product of incomplete combustion. In Mexico City, investigators suspect that benzene exposure might be elevated and may pose a risk to the population; however, no published data are available to confirm or disconfirm this suspicion. We, therefore, conducted a survey in 3 occupational groups in Mexico City. Forty-five volunteers who used portable passive monitors measured their personal exposure to benzene during a workshift. None of the participants smoked during the monitoring period. Benzene exposure was significantly higher among service-station attendants (mean = 359.5 microgram/m<sup>3</sup> [standard deviation = 170.4 microgram/m<sup>3</sup>]) than among the street vendors (83.7 and 45.0 microgram/m<sup>3</sup>, respectively) and office workers (45.2 microgram/m<sup>3</sup> and 13.3 microgram/m<sup>3</sup>, respectively). However, the benzene exposure levels observed among office workers were substantially higher than levels reported elsewhere for general populations. Our results highlight the need for more complete studies by investigators who should assess the potential benefits of setting environmental standards for benzene in Mexico.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

05-SEP-2000

(766)

**Remark:** Benzene production facility

**Source:** DSM Hydrocarbons B.V. Sittard

13-DEC-1996

**Remark:** Expositionsgefahr bei Herstellung, Lagerung, Umschlag, Transport.

**Source:** DEA Mineraloel AG Hamburg

13-DEC-1996

**Remark:** Nel sito produttivo di Scanzorosciate il Benzene (Bz) viene impiegato come materia prima per la produzione di Anidride Maleica (MA).  
Il Bz, stoccato in un serbatoio cilindrico verticale a tetto galleggiante della capacità pari a 6000 mc, viene inviato al relativo impianto di produzione in cui viene trasformato in MA mediante ossidazione catalitica.  
Nel sito produttivo sono in esercizio due unità di reazione sostanzialmente identiche denominate AM3 ed AM4 costituite da reattori a letto fisso.  
Il Bz non convertito viene inviato ad un impianto di abbattimento costituito da cinque adsorbitori a carbone attivo, in uscita dai quali ( a 30 metri dal suolo) la concentrazione in Bz nell' emissione è pari a 30 mg/mc (portata 81000 Nmc/h).

**Source:** Alusuisse Italia S.p.A. Scanzorosciate

13-DEC-1996

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.

Benzene is released into the UK environment as a result of its production and of oil refining processes in general, its use as a chemical intermediate (production of ethyl benzene, cyclohexane, phenol etc.), its presence in gasoline and the combustion of fossil fuels.

Studies in UK, Canada, Germany and USA estimate the relative contributions of these sources to be:

Vehicle emissions	79-85 %
Petrol distribution	2-6 %
Chemical industry	4-14 %
Petroleum refineries	1 %
Fossil fuel burning	2-5 %

Germany: In 1986, an extensive investigation programme on aromatic emissions from gasoline-powered cars was conducted by TUEV-Rheinland as part of a research project from DGMK/FVV/UBA. These measurements provide a good basis for more accurate estimates of car exhaust gas emission in the FRG. Using these results UBA estimated the total benzene emission from combustion engine driven vehicles, including diesel vehicles and motorcycles to be 42,000 t/a.

**Source:** Deutsche Shell Chemie GmbH Eschborn (95) (828) (1223)  
03-AUG-2000

**Country:** Italy

**Remark:** Benzene is used in three sites in Italy in alkylation processes to produce linear alkylbenzene in close system.

**Source:** ENICHEM AUGUSTA INDUSTRIALE Srl Milan  
13-DEC-1996

**Remark:** Benzene enters the environment from production, storage, transport, venting, and combustion of gasoline; and from production, storage, and transport of benzene itself. Other sources result from its use as an intermediate in the production of other chemicals, and as a solvent.. from its indirect production in coke, from nonferrous metal manufacture, ore mining, wood processing, coal mining and textile manufacture, from cigarette smoke..

manufacturing methods:

process from coal tar distillation

manufacturing methods:

Process high temperature carbonisation of coal to coke

Manufacturing methods: process petroleum-derived by catalytic reforming or by cracking of parafines

**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
13-DEC-1996

(551)

**Remark:**

## Air Quality Standards:

For :

United Kingdom: 500 ppb

U.S.A.: Louisiana 3.75 ppb ; Massachusetts : 0.0375 ppb

Germany: 4.7 ppb as from July '95 ; 3.1 ppb as from July '96.

Netherlands : 3.1 ppb as from Feb. '93

Benzene as reported here is produced by means of an extraction and fractionation process of benzene from a benzene rich gasoline stream in a petrochemical plant. EXXON CHEMICAL HOLLAND BV operates one manufacturing site for the production of benzene.

The production of benzene takes place in a completely closed system. The system will only be opened for maintenance, when empty ,after measurement of exposure concentrations and following strict control and working procedures.

Because of the carcinogenic properties of benzene, personel potentially exposed to benzene concentrations is continously monitored and guided.

## Consumer Exposure Literature:

## Note:

Following literature references have been included for sake of completeness. References report the potential sources of benzene exposure additional to sources as reported by our company for the benzene production - and end-use description in this HEDSET.

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## Note:

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EXXON CHEMICAL HOLLAND BV Botlek Rt

**Source:**  
13-DEC-1996

**Remark:** Benzene is used in closed systems.  
The occupational hazard is negligible versus the proposed TLV of 0.3 TWA, i.e. below/around 0.1 ppm (\*).

(\*Peakexposures may occur during shutdowns but also here, due to surveillance and the taken precautions (protective equipment/cleaning procedures), the exposure will be minimal. The proof of good working practise is the low levels of metabolite (phenol) found in the urine.

**Source:** Dow Benelux N.V. AA Terneuzen (306)  
13-DEC-1996

**Remark:** Processes:  
- Extraction by solvent (e.g. N-Methylpyrrolidone) of a cut predominantly C6 issued from steam cracking  
- Hydrodealkylation (thermic catalytic cracking)  
- Hydrodealkylation of benzols (issued from coal)

**Source:** Elf Atochem Paris La Defense 10  
13-DEC-1996

**Country:** United Kingdom

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.

Benzene is released into the UK environment as a result of its production and of oil refining processes in general; its use as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc.); its presence in gasoline; and combustion of fossil fuels.

Studies in the UK, Canada, Germany and the USA estimate the relative contributions of these sources to be :

Vehicle emissions	: 79-85%
Petrol distribution	: 2- 6%
Chemical industry	: 4-14%
Petroleum refineries	: 1%
Fossil fuel burning	: 2- 5%

**Source:** Shell UK Ltd. London (828) (1222)  
03-AUG-2000

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general to benzene.

Benzene is released into environment as :  
-vehicle emissions (79-85 %)  
-petrol distribution (2-6 %)  
-chemical industry (4-14 %)  
-fossil fuel burning (2-5 %)

Inhalation or skin contact when loading, unloading or using the product.

In case of accidental release, product may contaminate the environment.

**Source:** SHELL FRANCE Rueil Malmaison (828) (992) (1222)  
03-AUG-2000

**Source:** Ruetgers-Vft AG Duisburg  
13-DEC-1996

**Remark:** Aus C6- bzw. C7-Schnitt der Aromatendestillation des Aromizers und C7-Aromatenrücklauf der p-Xylol-Anlage werden in einem Mixer-Settler-Extraktor mittels Lösungsmittelgemisch (N-Methyl-Pyrrolidon/Glykol) im Gegenstrom Benzol und Toluol extrahiert, die Raffinatphase verläßt nach einer Wasserwäsche die Anlage. Im Lösungsmittelstripper wird unter Vakuum das Lösungsmittel von den Aromaten abgetrennt. Nach einer Bleicherdebehandlung wird Reinbenzol destillativ gewonnen. Lizenzgeber für das Verfahren ist die Lurgi Deutschland. Benzol wird im Ottokraftstofflager in Festdachtanks mit Schwimmdecke eingelagert. Pumpen, Kolonnen, Behälter und sonstige Apparate sind in Freibauweise errichtet und in Tassen aufgestellt. Sicherheitseinrichtungen wie z.B. Sicherheitsventile sind in ein Fackelsystem eingebunden. Die Verladung von Benzol erfolgt mit Abdichtkissen auf dem Dom des Kesselwagens, um Expositionen der Verloader gegenüber Benzol zu vermeiden.;

**Source:** PCK AG Schwedt Schwedt/Oder  
13-DEC-1996

**Remark:** Principal raw materials : crude benzole hydrogen.

Separation by distillation giving benzene.  
**Source:** Rhone-Poulenc Chemicals LTD WATFORD, HERTFORSHIRE  
13-DEC-1996

**Country:** United Kingdom

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.

Benzene is released into the UK environment as a result of its production and of oil refining processes in general; its use as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc.); its presence in gasoline; and combustion of fossil fuels.

Studies in the UK, Canada, Germany and the USA estimate the relative contributions of these sources to be :

Vehicle emissions : 79-85%

Petrol distribution : 2- 6%  
 Chemical industry : 4-14%  
 Petroleum refineries : 1%  
 Fossil fuel burning : 2- 5%

**Source:** Shell UK Limited, London (828) (1222)  
 03-AUG-2000

**Source:** REPSOL PETROLEO, S.A. MADRID (238) (239) (242)  
 03-AUG-2000

**Remark:** The product is only use by industrial profissionals jointly with a Material safety data sheet.;

**Source:** PETROLEOS DE PORTUGAL - PETROGAL,S.A. LISBOA  
 13-DEC-1996

**Remark:** Utilizzato in sistemi chiusi: esposizione possibile durante il campionamento.

**Source:** PRAOIL S.R.L. ASSAGO MI  
 13-DEC-1996

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.

**Source:** Shell Nederland Chemie B.V. Rotterdam (Pernis) (1222)  
 03-AUG-2000

**Remark:** Benzene is released into the UK environment as a result of its production and of oil refining processes in general, its use as a chemical intermediate (production of ethylbenzene, cyclohexane, phenol, etc), its presence in gasoline and the combustion of fossil fuels.

Studies in UK, Canada, Germany and USE estimate the relative contributions of these sources to be:

Vehicle emissions 79-85%  
 Petrol distribution 2- 6%  
 Chemical industry 4-14%  
 Petroleum refineries 1%  
 Fossil fuel burning 2- 5%

**Source:** Shell Nederland Chemie B.V. Rotterdam (Pernis) (828)  
 03-AUG-2000

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general to benzene.

Benzene is released into environment as :  
 -vehicle emissions (79-85 %)  
 -petrol distribution (2-6 %)

- chemical industry (4-14 %)
- fossil fuel burning (2-5 %)

Inhalation or skin contact when loading, unloading or using the product.

In case of accidental release, product may contaminate the environment.

**Source:** SHELL CHIMIE RUEIL MALMAISON (828) (992) (1222)  
03-AUG-2000

**Remark:** Emissions from petroleum refining, styrene manufacture.

**Source:** TOTAL PARIS LA DEFENSE  
13-DEC-1996

**Remark:** Benzene may be released to the environment during manufacturing and processing by the chemical industry. Major processing products are ethylbenzene (styrene, polystyrene plastics and synthetic rubber), cyclohexane (nylon), cumene/phenol (phenolic resins), nitrobenzene, chlorobenzenes and alkylbenzenes (detergents). To a significant amount benzene is further emitted into the atmosphere during combustion processes, mainly of fossile fuels (oil and coal), but also from wood burning and volcanoic activities. Important sources of airborne benzene regarding the exposure of individuals are automobile exhaust and cigarette smoke (see also section 3.2 below).

**Source:** Deutsche Shell Chemie GmbH Eschborn (56) (164) (171) (323) (324) (947)  
03-AUG-2000

**Remark:** In addition to natural benzene emissions, there is a range of man-made sources. In 1986, benzene emitted by gasoline-engined motor vehicles accounts for 80-85 percent of the total man-made benzene emissions. In addition, man-made benzene emissions arise from gasoline distribution (2.6 - 6%), petroleum refineries (0.3 - 1.5%), coke ovens (0.3 - 3.0%), chemical industry (1 - 11%), domestic heating (3 - 7%), solvent uses (1 - 4%). Benzene in motor gasoline in Europe is controlled to a maximum of 5% (by volume) though the current average is in the 3 - 3.5% range.

**Source:** Deutsche Shell Chemie GmbH Eschborn (231)  
06-JAN-1997

**Remark:** The annual Western European gasoline production was estimated at 120 million tonnes for 1995.

**Source:** Deutsche Shell Chemie GmbH Eschborn (230)  
06-JAN-1997

**Remark:** The smoke inhaled directly from a single cigarette is reported to contain 6 to 100 ug benzene. Using a value of 30 ug/cigarette and assuming 50% is exhaled unchanged, 20 cigarettes per day contributes 300 ug to the daily absorbed dose of a smoker.

The breath measurements carried out by the large scale US TEAM study (Total Exposure Assessment Methodology) identified smoking as the single most important source of benzene exposure. Smokers had a mean breath benzene concentration (15 ug/m<sup>3</sup>) almost 10 times the level observed in non-smokers (1.5-2 ug/m<sup>3</sup>).

The TEAM study identified a 50% increase in average benzene concentration in homes with one or more smokers (10.5 ug/m<sup>3</sup>) by comparison with homes without smokers (7 ug/m<sup>3</sup>). A similar finding was found in a German study: average benzene exposures were 10-12 ug/m<sup>3</sup> and 6-8 ug/m<sup>3</sup> in homes with and without smokers, respectively.

**Source:**  
06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(231)

**Remark:**

Based on the information available within the framework of the Risk Assessment Report (Existing Substances Regulation), it is concluded that exposure to benzene occurs in numerous of industries, e.g. in the chemical industry, in the petroleum refinery and in coking plants. Since benzene is a natural component of crude oil, it is an intrinsic constituent of certain refinery fractions, or it is formed during the refinery process in use today. As a result, benzene as a component of refinery products also ends up in products used at the workplace. Since 1989 the concentration of benzene in preparations is limited to 0.1 % (w/w). An exception is gasoline, which contains up to 1 % (v/v) benzene.

The assessment of occupational exposure is difficult because the legal conditions have changed recently and will change in the near future. E.g. in the past different OELs (Occupational Exposure Limits) were established, and the permitted concentration of benzene in gasoline has been reduced. Therefore, for many scenarios, exposure could not be assessed for the current situation at the workplace. Exposure levels based on data before 1995 do not reflect the current workplace situation.

Summary of exposure data:

Production and further processing, refinery:

Inhalation exposure: 3.5 mg/m<sup>3</sup> (8-h TWA, 95th percentile, based on data before 1995)

Dermal exposure: low (8-h TWA, expert judgement for proper use of suitable gloves); 420 mg/person/day (8-h TWA, model estimate for use of unsuitable gloves)

Coking plant, by-product recovery:

Inhalation exposure: 15.5 mg/m<sup>3</sup> (8-h TWA, highest measurement, 1990-1991)

Dermal exposure: 420 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Production of perfumes (use of pure benzene):

Inhalation exposure: 84 mg/m<sup>3</sup> (8-h TWA, 90th percentile, 1987-1995)

Dermal exposure: 420 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Production of formulations (use of solvents, benzene content unknown, assumption: 1 %):

Inhalation exposure: 0.15 mg/m<sup>3</sup> (8-h TWA, highest measurement, 1987-1999)

Dermal exposure: 4.2 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Distribution of gasoline (content: 1 %):

Inhalation exposure: 6.8 mg/m<sup>3</sup> (8-h TWA, 90th percentile, for workplaces without VR, 1999-2000); 1.26 mg/m<sup>3</sup> (8-h TWA, 90th percentile, for workplaces with VR, 1999-2000)

Dermal exposure: 4.2 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Automobile industry, mechanic engineering, car repair and recycling (content: 1 %):

Inhalation exposure: 2.25 mg/m<sup>3</sup> (8-h TWA, modified model estimates, based on data before 1995, the exposure level represent the current exposure situation)

Dermal exposure: 8.4 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Service stations, handling of gasoline (content: 1 %):

Inhalation exposure: 0.5 mg/m<sup>3</sup> (8-h TWA, 90th percentile, for workplaces without VR, based on data before 2000); 0.1 mg/m<sup>3</sup> (8-h TWA, 90th percentile, for workplaces with VR, 1999-2000)

Dermal exposure: 0.4 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Cleaning of crude benzene or gasoline tanks (content: 75 % benzene):

Inhalation exposure: 67.7 mg/m<sup>3</sup> (8-h TWA, 95th percentile, based on data before 2000)

Dermal exposure: 1575 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Cleaning of heating oil tanks:

Inhalation exposure: 0.44 mg/m<sup>3</sup> (8-h TWA, highest result, 2000)

Dermal exposure: negligible (8-h TWA, expert judgement based on the low concentration of benzene in heating oil; < 1 mg/person/day)

Use of formulations with residual benzene (content: < 0.1 % benzene), e.g. adhesives, paints:

Inhalation exposure: 1 mg/m<sup>3</sup> (8-h TWA, 95th percentile, 1991-1995)

Dermal exposure: 6.5 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Tire retreading, plastics, inter alia using adhesives, content of benzene limited to 0.1 %:

Inhalation exposure: 2.7 mg/m<sup>3</sup> (8-h TWA, highest result,

based on data before 1991)  
Dermal exposure: 0.4 mg/person/day (8-h TWA, model estimate for the unprotected worker)

Foundries (benzene released as a decomposition product):  
Inhalation exposure: 5.4 mg/m<sup>3</sup> (8-h TWA, 95th percentile, for workplaces without LEV, 1991-1995); 1.6 mg/m<sup>3</sup> (8-h TWA, 95th percentile, for workplaces with LEV, 1991-1995)  
Dermal exposure: low (8-h TWA, rough estimation, contact with contaminated surfaces)

Dermal exposure is exclusively assessed according to the EASE model (Estimation and Assessment of Substance Exposure). In the case of benzene and gasoline, the predominant effect reducing dermal exposure is the high volatility of the substances (vapour pressure of benzene: 99.7 hPa and of gasoline: 50 - 900 hPa.) which leads to considerable low retention times of the substances on the skin or on the protective gloves. An evaporation time of up to 10 seconds (order of magnitude) is calculated and given in addition to the EASE estimates. This exposure reducing effect cannot be considered if workers have continuous direct contact with the substances, e.g. dipping hands into the substances. In case of occlusive conditions the retention time may be prolonged to a few minutes. Non-occlusive exposure is regarded as predominant. For further details, see <http://ecb.jrc.it/existing-chemicals> (public documents - III. Risk Assessment - finalised comprehensive risk assessment reports, the summary risk assessment reports - R063).

8-h TWA : 8 hour time weighted average  
LEV: local exhaust ventilation  
VR: vapour recovery

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
07-JAN-2005

**Remark:** Utilizzato in sistemi chiusi: esposizione possibile durante il campionamento.

**Source:** ENI SpA - Divisione Refining & Marketing ROMA  
20-MAY-1994

**Remark:** Based on extensive studies in the USA, it appears that facilities manufacturing chemicals, drinking water, food and beverages, and petroleum refining operations play only a minimal role in the total exposure of the general population to benzene.  
Benzene is released into the UK environment as a result of its production and of oil refining processes in general, its use as a chemical intermediate (production of ethyl benzene, cyclohexane, phenol etc.), its presence in gasoline and the combustion of fossil fuels.  
Studies in UK, Canada, Germany and USA estimate the relative

contributions of these sources to be:

Vehicle emissions	79-85 %
Petrol distribution	2-6 %
Chemical industry	4-14 %
Petroleum refineries	1 %
Fossil fuel burning	2-5 %

Germany: In 1986, an extensive investigation programme on aromatic emissions from gasoline-powered cars was conducted by TUEV-Rheinland as part of a research project from DGMK/FVV/UBA. These measurements provide a good basis for more accurate estimates of car exhaust gas emission in the FRG. Using these results UBA estimated the total benzene emission from combustion engine driven vehicles, including diesel vehicles and motorcycles to be 42,000 t/a.

**Source:** Deutsche Shell Chemie GmbH Eschborn (95) (829) (1224)  
25-MAY-1994

### 1.11 Additional Remarks

**Remark:** By barge (sometimes by roadtanker)  
**Source:** DSM Hydrocarbons B.V. Sittard  
13-DEC-1996

**Remark:** Entsorgung: Rückgewinnung durch Destillation.  
Transportvorschriften: Kl.3; Ziff.3b); Kemmlerzahl 33;  
UN-Nr.1114.  
**Source:** DEA Mineraloel AG Hamburg  
13-DEC-1996

**Remark:** Disposal: reprocess if feasible, may be incinerated.  
Transport:by road, rail and sea. UN No 1114 Hazchem 3WE  
**Source:** Conoco Limited Warwick  
13-DEC-1996

**Remark:** Smaltimento.  
Il Benzene (Bz) può essere inviato all' incenerimento in inceneritori autorizzati.  
Il Bz, altamente infiammabile e volatile, deve essere trattato con estrema cautela nella fase di accensione.  
**Source:** Alusuisse Italia S.p.A. Scanzorosciate (22)  
13-DEC-1996

**Remark:** Trasporto.  
Il Benzene (Bz) arriva al sito produttivo di Scanzorosciate mediante autobotti e vagoni cisterna ferroviari.  
Attualmente sono previsti complessivamente da 5 a 6 arrivi giornalieri.  
Il Bz è soggetto alla regolamentazione nazionale ed internazionale relativa al trasporto di merci pericolose.  
Ai fini del trasporto stradale e ferroviario è classificato nella categoria 3 relativa ai materiali molto infiammabili

all' ordianle 3b.  
**Source:** Alusuisse Italia S.p.A. Scanzorosciate  
13-DEC-1996

**Remark:** Disposal options:  
Recover or recycle if possible. Otherwise: incineration.

Transport classification:  
UN number: 1114  
IMO class/Packing group: 3.2/II  
Marine pollutant: No  
IMO symbol: Flammable liquid  
IMO proper shipping name: benzene  
ADR/RID class/item: 3/3 b  
ADR/RID symbol: Flammable liquid  
ADR/RID Kemler number: 33  
ADR/RID packing group: 2  
ADR/RID proper shipping name: benzene  
ICAO class: 3  
ICAO packing group: II  
ICAO proper shipping name: benzene

TA-Luft (1986):  
Max. emission of 5 mg/m<sup>3</sup> (carcinogenic substances class III)

Stoerfall-Verordnung (1991):  
Substance number 39

Verordnung ueber brennbare Fluessigkeiten (1990):  
A I

**Source:** Deutsche Shell Chemie GmbH Eschborn  
13-DEC-1996

**Remark:** La produzione della sostanza è terminata nel 1993

**Source:** Kuwait Raffinazione e Chimica Napoli  
13-DEC-1996

**Remark:** The primary routes of exposure are inhalation of contaminated air, especially in areas with high traffic, and in the vicinity of gasoline service stations and consumption of contaminated drinking water.

For late model cars it has been estimated that over 90% of automotive benzene comes from exhaust and less than 10% from fuel evaporation.. (Re: Verschueren, Handbook of environm. data of organic compound, 2Ed. NY, Van Nostrand Reinhold Co, 1983, 238).

In 1976, an estimated 1.3 billion pounds of benzene were released into the atmosphere from 132 million stationery and mobile sources...(in USA). (Re: DHHS/NTP, Fourth Annual Report on Carcinog., p.35, 1985).

**Source:** ENICHEM SYNTHESIS S.p.A. MILANO  
13-DEC-1996

**Remark:** Benzene as reported here is only transported by ship or vessel.  
Routes for disposal: benzene may be disposed of by controlled incineration, re-used as a blending component for fuel or can be recovered by distillation.  
Transport information:  
By land: (railroad/road, such as RID/ADR):  
ADR/RID Class :3,3b ; Danger Number: 33 ; Danger Label :3  
Substance ID number: 1114  
By inland waterways:  
ADN/R Class: IIIa,1.a ; ADN/R Category :K1n  
By Sea (IMDG):  
UN Number:1114 ; IMO Class: 3.2 ; IMDG Code : 3185 ; MFAG Number : 312 ; Packing group: II ; Risk Label : 3

**Source:** EXXON CHEMICAL HOLLAND BV Botlek Rt  
13-DEC-1996

**Remark:** no additional remarks  
**Source:** Dow Benelux N.V. AA Terneuzen  
13-DEC-1996

**Remark:** Disposal Options  
  
Recover or recycle if possible. Otherwise incinerate.  
Transport Conventions  
  
IMO/IMDG  
  
Class : 3.2  
UN No. : 1114  
Packing Group : II  
Page No. : 3185  
Marine Pollutant : No  
Transport Information  
  
Transported by : Sea  
Frequency : 4/5 times per month

**Source:** Shell UK Ltd. London  
13-DEC-1996

**Remark:** Disposal options : Recover or recycle if possible. Otherwise : incineration.  
  
Avoid electrostatic discharge generation.  
Earth all equipment.  
Avoid splash filling.  
Do not use compressed air or oxygen for filling, discharging or handling.  
Use a vapour recovery system.  
If positive displacement pumps are used, these must be fitted with a non-integral pressure relief valve.  
Restrict line velocity during the pumping in order to avoid generation of electrostatic discharge.

Do not smoke. Avoid naked flames. Remove ignition sources.  
Avoid sparks.

## Transport Information

UN number : 1114  
Class : 3  
Packing Group : II  
Proper Shipping Group : Benzene

Sea (IMO)  
Class/Packing group : 3.2/II  
Marine Pollutant (Y/N) : No  
Symbol : Flammable liquid

Rail/Road (RID/ADR)  
Class : 3  
Item : 3(b)  
Symbol : Flammable liquid  
Kemler Plate : 33/1114

Air (IATA/IACO)  
Class : 3  
Symbol : Flammable liquid  
SHELL FRANCE Rueil Malmaison

**Source:**  
13-DEC-1996

**Remark:** Beförderung in Eisenbahnkesselwagen  
Hinweise zur Entsorgung:  
Empfehlung: Durch Destillation zurückgewinnen, in geeigneter  
Verbrennungsanlage vernichten oder als Sondermüll entsorgen.

**Source:** ÖMV Deutschland GmbH Burghausen (991)  
03-AUG-2000

**Remark:** Die Abgabe an Dritte darf nur erfolgen bei Beachtung der -  
GGVO  
- Verordnung über Verbote und Beschränkungen des  
Inverkehrbringens gefährlicher Stoffe, Zubereitungen und  
Erzeugnisse nach dem Chemikaliengesetz  
(Chemikalienverbotsverordnung-ChemVerbotsV) vom 14. Oktober  
1993 BGBl. I S.1720

**Source:** PCK AG Schwedt Schwedt/Oder  
13-DEC-1996

**Remark:** Wasserschadstoff:  
Wassergefährdungsklasse 3  
KBwS-Einstufung

Luftschadstoff:  
Benzol ist im Anhang II der Störfallverordnung  
unter Nr.39 sowie unter Ziffer 2.3 der TA-Luft  
mit Klasse III zu finden.

**Source:** Sächsische Olefinwerke GmbH Böhlen  
13-DEC-1996

**Remark:** Benzene delivered in up to 20 tonnes stainless steel road tankers. Offtake by pump. Benzene in 181 kg drums delivered on a flatbed lorry (+ 205 l. packages).

UK Road :

Hazchem code : 3 WE  
Packing group II - ID No 1114.

RID - ADR -class 3 Hem 3 (b)

IMO : IMDG P 3185

ICAO : Passenger 305 (5 litres). Cargo : 307 (60 litres).

- Water pollution :  
-----  
UK legislation : Environmental pollution Act 1990  
monitored by HMIP.  
Categorie de danger WGK : 0

- Major accidents hazards :  
UK : complies to (CIMAH) Control of Industrial Major  
Accidents Hazards Regulations.

- Air pollution : Environmental pollution Act 1990 (part. I)  
monitored by HMIP.

**Source:** Rhone-Poulenc Chemicals LTD WATFORD, HERTFORDSHIRE  
13-DEC-1996

**Remark:** Disposal Options

Recover or recycle if possible. Otherwise incinerate.  
Transport Conventions

IMO/IMDG

Class : 3.2  
UN No. : 1114  
Packing Group : II  
Page No. : 3185  
Marine Pollutant : No

Transport Information

Transported by : Sea  
Frequency : 4/5 times per month

**Source:** Shell UK Limited, London  
13-DEC-1996

**Remark:** DISPOSAL METHODS :

Small spills : take up with sand or other noncombustible adsorbent material and place into containers for later disposal.

Large spills : avoid dispersion of liquid with barriers or

mechanicals containment.

Surplus : disposal methods are combustion or incineration.

HANDLING : Wear self contained breathing apparatus to avoid vapour inhalation. Safety goggles, gloves and impervious suits are recommended when there is risk of contact with the material. Exposure monitoring at work. Do not smoke and eliminate all sources of ignition from areas where the material is stored, handled or used. Never exposed stored material in containers to open air. Product transfer must be done in earthed, airtight conducts. Used antisparking equipment and tools.

STORED : Protect containers against physical damage or fire. Containers properly labelled and sealed, placed in cool and well ventilated areas.

TRANSPORT :

UN No. : 1114

Hazard Identification No. : 33

ADR/RID : 3, Item 3B

IMDG Class : 3.2

IATA-DGR : 3

Packing group : II

**Source:**  
13-DEC-1996

REPSOL PETROLEO, S.A. MADRID

**Remark:**

Transport information :

UN number 1114

RPE 3, 3° b) ; 33 ; 3

IMO 3.2

**Source:**  
03-AUG-2000

PETROLEOS DE PORTUGAL - PETROGAL, S.A. LISBOA

**Remark:**

DISPOSAL OPTIONS

Recover or recycle if possible. Otherwise :incineration.

TRANSPORT INFORMATION

UN Number: 1114

Class: 3

Packing group: II

Proper Shipping Name: Benzene

Sea (IMO)

Class: 3.2

Packing Group: II

Symbol: Flammable liquid

Marine Pollutant (Y/N): No

Rail/Road (RID/ADR)

Class: 3

Item: 3(b)

Symbol: Flammable liquid  
Kemler Plate: 33/1114

Air (IATA/ICAO)

Class: 3

Packing Group: II

Symbol: Flammable liquid

**Source:**

13-DEC-1996

Shell Nederland Chemie B.V. Rotterdam (Pernis)

**Remark:**

Disposal options : Recover or recycle if possible. Otherwise : incineration.

Avoid electrostatic discharge generation.

Earth all equipment.

Avoid splash filling.

Do not use compressed air or oxygen for filling, discharging or handling.

Use a vapour recovery system.

If positive displacement pumps are used, these must be fitted with a non-integral pressure relief valve.

Restrict line velocity during the pumping in order to avoid generation of electrostatic discharge.

Do not smoke. Avoid naked flames. Remove ignition sources.

Avoid sparks.

Transport Information

UN number : 1114

Class : 3

Packing Group : II

Proper Shipping Group : Benzene

Sea (IMO)

Class/Packing group : 3.2/II

Marine Pollutant (Y/N) : No

Symbol : Flammable liquid

Rail/Road (RID/ADR)

Class : 3

Item : 3(b)

Symbol : Flammable liquid

Kemler Plate : 33/1114

Air (IATA/IACO)

Class : 3

Symbol : Flammable liquid

**Source:**

13-DEC-1996

SHELL CHIMIE RUEIL MALMAISON

**Remark:**

INFORMATIONS RELATIVES AU TRANSPORT

Réglementations internationales:

par voies terrestres: classe 3, énumération: 3°b),  
étiquette: 3, code danger: 33, code matière: 1114

**Source:** par voie maritime: classe: 3.2, page: 3185, étiquette:3  
TOTAL PARIS LA DEFENSE  
13-DEC-1996

**Remark:** Analytical methods for the determination of benzene in environmental samples can be summarized as follows:  
Air: preconcentration by passing through a solid sorbent (Tenax resin, silica gel or activated carbon), thermal desorption or desorption by a solvent (e.g. CS<sub>2</sub>), gaschromatographic detection with a flame-ionization detector(FID) or a mass-selective detector (MSD). Detection limits are usually in the low ppb (ug/m<sup>3</sup>) range.  
Water, soil, sediment, food: isolation by purge-and-trap methods, purging of the sample by an inert gas (e.g. N<sub>2</sub>), gaschromatographic detection (detectors see above). Detection limits are usually in the low to sub ug/l range (water) and as low as 1 ng/kg (soil, sediment, food), respectively. In the latter case, recoveries and precision seem to be frequently low.

**Source:** Deutsche Shell Chemie GmbH Eschborn (324)  
06-JAN-1997

**Remark:** Disposal options:  
Recover or recycle if possible. Otherwise: incineration.

Transport classification:  
UN number: 1114  
IMO class/Packing group: 3.2/II  
Marine pollutant: No  
IMO symbol: Flammable liquid  
IMO proper shipping name: benzene  
ADR/RID class/item: 3/3 b  
ADR/RID symbol: Flammable liquid  
ADR/RID Kemler number: 33  
ADR/RID packing group: 2  
ADR/RID proper shipping name: benzene  
ICAO class: 3  
ICAO packing group: II  
ICAO proper shipping name: benzene

TA-Luft (1986):  
Max. emission of 5 mg/m<sup>3</sup> (carcinogenic substances class III)

Stoerfall-Verordnung (1991):  
Substance number 39

Verordnung ueber brennbare Fluessigkeiten (1990):  
A I

**Source:** Deutsche Shell Chemie GmbH Eschborn  
25-MAY-1994

## 2.1 Melting Point

**Value:** = 5 degree C  
**Decomposition:** no at degree C  
**Sublimation:** yes

**Source:** REPSOL PETROLEO, S.A. MADRID  
**Test substance:** Sublimation at -30 to 5 deg C.  
**Reliability:** (4) not assignable  
01-AUG-2000

**Value:** = 5.5 degree C  
**Decomposition:** no at degree C

**Method:** other  
**GLP:** no data

**Remark:** No further details were available.  
**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201) (433) (531) (575) (767) (856)

**Value:** = 5.5 degree C

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
03-AUG-2000 (73)

**Value:** 5.5 degree C  
**Decomposition:** no at degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (60) (165) (362) (434) (688) (1010)

**Value:** = 5.5 degree C  
**Decomposition:** no at degree C

**Method:** other  
**GLP:** no data

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (983)

**2.2 Boiling Point****Value:** = 80 degree C**Source:** BASF AG Ludwigshafen**Reliability:** (4) not assignable

02-AUG-2000

(73)

**Value:** = 80 degree C**Source:** REPSOL PETROLEO, S.A. MADRID**Reliability:** (4) not assignable

01-AUG-2000

**Value:** = 80.1 degree C at 1.013 hPa**Decomposition:** no**Method:** other**GLP:** no data**Remark:** No further details were available.**Source:** BP Chemicals Ltd LONDON**Reliability:** (2) valid with restrictions

03-AUG-2000

(201) (433) (531) (575) (767) (856)

**Value:** 80.1 degree C at 1013.25 hPa**Decomposition:** no**Method:** other: no further information**GLP:** no data**Source:** Deutsche Shell Chemie GmbH Eschborn**Reliability:** (2) valid with restrictions

02-AUG-2000

(60) (165) (362) (688) (1010)

**Value:** 80.1 degree C at 1.013 hPa**Method:** other**GLP:** no data**Source:** German rapporteur**Reliability:** (2) valid with restrictions**Flag:** Risk Assessment

02-AUG-2000

(983)

### 2.3 Density

**Type:** relative density  
**Value:** = .8787 at 15 degree C

**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (4) not assignable  
01-AUG-2000

**Type:** relative density  
**Value:** = .879 at 15 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
01-AUG-2000 (165)

**Type:** relative density  
**Value:** = .8765 at 20 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
01-AUG-2000 (362) (688)

**Type:** density  
**Value:** = .8788 g/cm<sup>3</sup> at 20 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
01-AUG-2000 (60)

**Type:** relative density  
**Value:** = .879 at 20 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
01-AUG-2000 (1010)

**Type:** density  
**Value:** = .879 g/cm<sup>3</sup> at 20 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
01-AUG-2000 (362)

**Type:** relative density  
**Value:** = .879 at 20 degree C

**Method:** other  
**GLP:** no data

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (983)

**Type:** density  
**Value:** = .879 g/cm<sup>3</sup>

**Method:** other  
**GLP:** no data

**Remark:** Test conducted at 15-20°C. No further details were available.

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201) (531) (767) (856)

**Type:** density  
**Value:** = .88 g/cm<sup>3</sup>

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

### 2.3.1 Granulometry

-

### 2.4 Vapour Pressure

**Value:** = 10 hPa at 10 degree C

**Remark:** Vapour pressure: 92 mmHg at 20 C  
**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (4) not assignable  
02-AUG-2000

**Value:** 99.7 hPa at 20 degree C

**Method:** other (calculated): no further information  
**GLP:** no data

**Remark:** temperature (degree C) vapour pressure (hPa)  
0 34.66  
10 59.65  
30 157.8  
40 241.9

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (60) (362)

**Value:** = 100 hPa at 20 degree C

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

**Value:** = 126.9 hPa at 25 degree C

**GLP:** no data

**Remark:** Value given converted from cited value of 95.18 Torr. No further details available. Other vapour pressures of 100 mmHg at 26.1°C (converts to 133.32 hPa; HSDB, 1993) and 40 mmHg at 7.6°C (converts to 53.33 hPa; OHMTADS, 1987) have been reported.

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (311) (531) (575) (856)

**Value:** = 200 hPa at 38 degree C

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

## **2.5 Partition Coefficient**

**log Pow:** = 1.83

**Method:** other (measured): HPLC  
**GLP:** no data

**Remark:** Test procedure not described in detail  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test condition:** room temperature; substance concentration: ca. 1 g/l;  
reversed phase analytical column; mobile phase:  
methanol/water; UV detection  
**Test substance:** commercial product (purity not given)  
**Reliability:** (4) not assignable

02-AUG-2000 (314)

**log Pow:** = 2.11**Method:** other (measured): batch tests with gaschromatographic determination of substance concentration**GLP:** no data**Remark:** No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail**Source:** Deutsche Shell Chemie GmbH Eschborn**Test condition:** Room temperature**Test substance:** commercial product (spectrograde, purity not given)**Reliability:** (2) valid with restrictions

02-AUG-2000 (601)

**log Pow:** = 2.13**GLP:** no data**Remark:** The log Pow (octanol-water partition coefficient) was reported as above. No further details on test method were available.**Source:** BP Chemicals Ltd LONDON**Reliability:** (2) valid with restrictions

03-AUG-2000 (194) (195) (531) (626) (680) (854) (932)

**log Pow:** = 2.13**Source:** REPSOL PETROLEO, S.A. MADRID**Reliability:** (4) not assignable

02-AUG-2000

**log Pow:** = 2.13 at 25 degree C**Method:** other (measured): HPLC**GLP:** no data**Source:** Deutsche Shell Chemie GmbH Eschborn**Reliability:** (2) valid with restrictions**Flag:** Risk Assessment

02-AUG-2000 (996)

**log Pow:** = 2.14**Source:** BASF AG Ludwigshafen**Reliability:** (4) not assignable

02-AUG-2000 (73)

**log Pow:** 2.15  
**Method:** other (measured)  
**GLP:** no data  
**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (474) (588) (1207)

**log Pow:** 2.19  
**Method:** other (measured)  
**GLP:** no data  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (1204)

### 2.6.1 Solubility in different media

**Solubility in:** Water  
**Value:** = 1.8 g/l at 25 degree C  
**Method:** other  
**GLP:** no data  
**Remark:** No further details were available on this study.  
**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (575) (1087)

**Solubility in:** Water  
**Value:** = 1.8 g/l at 20 degree C  
**Remark:** Mixible with many organic solvents.  
**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

**Solubility in:** Water  
**Value:** = .06 vol%  
**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (3) invalid  
02-AUG-2000

**Solubility in:** Water  
**Value:** ca. 1.88 g/l at 23.5 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (165)

**Solubility in:** Water  
**Value:** = 1.8 g/l at 25 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (374) (434)

**Solubility in:** Water  
**Value:** = 1.71 g/l at 25 degree C

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (4) not assignable  
02-AUG-2000 (163)

**Solubility in:** Water  
**Value:** = 1.78 g/l at 25 degree C

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (719)

**2.6.2 Surface Tension**

**Test type:** other  
**Value:** = 28.9 mN/m

**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
03-AUG-2000 (201) (531) (1241)

**Test type:** other  
**Value:** = 29 mN/m

**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (1009)

### **2.7 Flash Point**

**Value:** -11.1 degree C

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (434)

**Value:** = -11 degree C  
**Type:** closed cup

**Method:** other  
**GLP:** no data

**Remark:** No further details were available.  
**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201) (492) (767)

**Value:** = -11 degree C

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

**Value:** = -11 degree C  
**Type:** closed cup

**Method:** other  
**Year:** 1983  
**GLP:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (1122)

**Value:** -11 degree C  
**Type:** closed cup

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (60) (165) (362) (1010)

**Value:** -11 degree C  
**Type:** closed cup

**Method:** other: DIN 51755  
**GLP:** no data

**Source:** German rapporte  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (185)

### 2.8 Auto Flammability

**Value:** = 555 degree C

**Method:** other: DIN 51794  
**GLP:** no data

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (185)

**Value:** = 562 degree C

**Method:** other  
**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (492) (531) (811)

**Value:** = 562 degree C at 1013.25 hPa

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (811) (1010)

**Value:** = 580 degree C

**Method:** other  
**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (856)

**Value:** = 590 degree C

**Method:** other: see reference  
**Year:** 1986  
**GLP:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (811)

**Value:** = 591 degree C

**Method:** other  
**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201)

**Value:** 595 degree C at 1013.25 hPa

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (362)

**Value:**

**Remark:** Ignition temperature: 555 Grad C  
**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (73)

**2.9 Flammability**

**Result:** flammable

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (4) not assignable  
02-AUG-2000 (362) (1010)

**Result:** highly flammable

**Method:** other  
**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201) (492) (767)

**Result:** highly flammable

**Method:** other: Dir. 67/548/EEC  
**Year:** 1967  
**GLP:** no data

**Remark:** Lower Flammability Limit : 1.3%  
Upper Flammability Limit : 7.1%

**Source:** REPSOL PETROLEO, S.A. MADRID  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (811)

**Result:** highly flammable

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (165)

**Result:** highly flammable

**GLP:** no data

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (185)

### 2.10 Explosive Properties

**Result:** explosive under influence of a flame

**Method:** other  
**GLP:** no data

**Remark:** The vapour may explode if ignited in an enclosed area. It may form an explosive mixture with air.

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (2) valid with restrictions  
03-AUG-2000 (201) (856)

**Result:** not explosive

**Method:** other  
**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
**Reliability:** (4) not assignable  
02-AUG-2000 (139)

**Result:** not explosive

**Method:** other: no further information  
**GLP:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (4) not assignable  
02-AUG-2000 (138)

**Result:** not explosive

**Method:** other: no further data  
**GLP:** no data

**Result:** Pure liquid benzene is stable; Morrison and Boyd (1973) comment on the "unusual stability of the benzene ring," which refers to higher chemical stability than other hydrocarbons of similar molecular weight.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (795)

**Result:** not explosive

**GLP:** no data

**Source:** German rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-AUG-2000 (185)

**GLP:** no data

**Remark:** The lower and upper explosive limits in air have been reported to be 1.2 and 8 % respectively.

**Source:** BASF AG Ludwigshafen  
**Reliability:** (4) not assignable  
02-AUG-2000 (74)

**GLP:** no data

**Remark:** The lower and upper explosive limits in air have been reported to be 1.4 and 8.0 % respectively.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
**Reliability:** (2) valid with restrictions  
02-AUG-2000 (142) (1008)

**GLP:** no data

**Remark:** The lower and upper explosive limits in air have been reported to be 1.5 and 8 % (v/v) respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
REPSOL PETROLEO, S.A. MADRID

**Reliability:** (2) valid with restrictions

02-AUG-2000

(1010)

**GLP:** no data

**Remark:** The lower and upper explosive limits in air have been reported to be 1.4 and 6.7 % (v/v) respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Reliability:** (2) valid with restrictions

02-AUG-2000

(362)

### 2.11 Oxidizing Properties

**Result:** no oxidizing properties

**Method:** other: no further information

**GLP:** no data

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

02-AUG-2000

(138)

**Result:** no oxidizing properties

**GLP:** no data

**Source:** German rapporteur

02-AUG-2000

(1)

### 2.12 Dissociation Constant

-

### 2.13 Viscosity

-

### 2.14 Additional Remarks

**Remark:** Benzene floats on water.

**Source:** BP Chemicals Ltd LONDON

13-DEC-1996

(201)

- Remark:** Liquid-water interfacial tension: 35 dynes/cm at 20 degree C (0.035 N/m).  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (201)
- Remark:** Latent heat of vaporisation: 169 Btu/lb (94.1 cal/g;  $3.94 \times 10^5$  J/kg).  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (201) (531)
- Remark:** Heat of combustion: -17460 Btu/lb (-9698 cal/g;  $-406 \times 10^5$  J/kg).  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (201) (531)
- Remark:** Heat of fusion: 30.45 cal/g (127.4 J/g; 9951 J/mol).  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (201) (531)
- Remark:** Heat capacity: 135.6 J/mol-degree K at 1 atm (liquid); 81.6 J/mol-degree K at 1 atm (gas).  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (531)
- Remark:** Viscosity: 0.6468 mPa's at 20 degree C.  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (531)
- Remark:** % in saturated air: 13.15 at 26 degree C and 1 atm.  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (531)
- Remark:** Miscible with alcohol, chloroform, ether, carbon disulphide, acetone, oils, carbon tetrachloride, glacial acetic acid.  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (492) (531)
- Remark:** Dangerous reaction with acids, oxidising agents, bromine, chlorine, iodine, sulphur (liquid)  
Viscosity: 0.652 mPa.s at 20 degree C  
**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (73)

- Remark:** Stability: Flammable and combustible.  
Conditions to avoid : Exposure to heat, sparks, static electricity or flames.  
Incompatibilities: Strong oxidants.  
Hazardous decomposition/combustion products: CO and toxic vapours in case of incomplete combustion.  
Extinguishing agents: Foams, dry chemicals, CO<sub>2</sub>. Water may be ineffective but water should be used to keep fire-exposed containers cool.  
Special hazards: Vapours may travel to a source of ignition and flash back. Runoff to drains or sewer may create fire and explosions hazards.
- Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996
- Remark:** undissolved benzene floats on water  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (434)
- Remark:** heat of vaporisation: 435.0 J/g at 20 degree C  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (362)
- Remark:** heat of combustion: -3725.3 kJ/mol (-47760 J/g)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (362)
- Remark:** heat of fusion: 125.9 J/g  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (362)
- Remark:** viscosity: 0.654 mPa x s at 20 degree C  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (362)
- Remark:** miscible with ethanol, diethylether, carbondisulfide, acetone, tetrachlormethane, acetic acid  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (688) (1010)
- Remark:** Henry's Law Constant: 2.67E-3 atm x m<sup>3</sup>/mol at 10 degree C  
(270.5 Pa x m<sup>3</sup>/mol)  
5.5E-3 atm x m<sup>3</sup>/mol at 25 degree C  
(557.3 Pa x m<sup>3</sup>/mol)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (722)

- Remark:** Henry's Law Constant: 533-570 Pa x m<sup>3</sup>/mol at 25 degree C  
(experimental value: 562 Pa x m<sup>3</sup>/mol; recommended value:  
550Pa x m<sup>3</sup>/mol; compilation of literature data)
- Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (723)
- Remark:** odour threshold (water): 0.17 mg/l  
odour threshold (air): 39 mg/m<sup>3</sup> (12 ppm)
- Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (30)
- Remark:** odour threshold (air): 4.68 ppm (15.2 mg/m<sup>3</sup>)
- Source:** Deutsche Shell Chemie GmbH Eschborn
- Test substance:** highest purity commercially available product from large  
scale production
- 06-JAN-1997 (681)
- Remark:** saturation vapour concentration: 320 g/m<sup>3</sup> at 20 degree C  
489 g/m<sup>3</sup> at 30 degree C
- Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (60)

### 3.1.1 Photodegradation

**Type:** air  
**Light source:** other  
**Conc. of subst.:** at 24 degree C

#### INDIRECT PHOTOLYSIS

**Sensitizer:** O3  
**Conc. of sens.:** 3000000000000 molecule/cm<sup>3</sup>  
**Rate constant:** = .000000000000000000000001 cm<sup>3</sup>/(molecule \* sec)  
**Degradation:** = 50 % after 105 year

**Method:** other (calculated)  
**GLP:** no data  
**Test substance:** no data

**Result:** In an urban atmosphere, the half-life for the reaction of benzene with ozone was calculated to be 105 years. In a rural atmosphere, the half-life was found to be 327 years, using an atmospheric concentration for ozone of  $9.6 \times 10^{11}$  molecules/cm<sup>3</sup>. Consideration of the reaction with ozone in isolation from other sensitizer species is not a realistic scenario for benzene atmospheric fate.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The light source was chemiluminescence. The rate constant used was that of Pate C.T. et al. J. envir. Sci. Hlth A11, 1-10, 1976 and the sensitizer concentration that of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. McGraw-Hill Book Company, New York, 1982.

**Reliability:** (4) not assignable  
15-MAR-2004 (54) (184)

**Type:** air  
**Conc. of subst.:** .013 mg/l

#### INDIRECT PHOTOLYSIS

**Sensitizer:** O3  
**Conc. of sens.:** .001 mg/l

**Method:** other (measured)  
**GLP:** no data  
**Test substance:** no data

**Result:** Very little aerosol was formed when benzene reacted with ozone in the dark.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** A light scattering photometer was used. No further details were available.

**Reliability:** (4) not assignable  
15-MAR-2004 (184) (447)

**Type:** air  
**Light source:** other: high pressure mercury lamp  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** O3

**Method:** other (measured): determination of degradation products in a free flow system  
**Year:** 1993  
**GLP:** no data  
**Test substance:** other TS: commercial product (purity 99.5%)

**Result:** Quantum yield (254 nm): 0.05 +/- 0.02; from this result the authors conclude that chain reaction steps are not important for ozone-sensitized benzene photooxidation.  
Main degradation products: phenol, biphenyl, benzoic aldehyde, maleic acid anhydride, p-benzoquinone, toluene. Phenol seemed to be the by far abundant component.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** room temperature; substance concentrations: 0.17 and 1.3 hPa; ozone concentration: <= 150 ppm; irradiation time: 3-8 h; product accumulation in a cold trap; GC-MS analysis

**Reliability:** (2) valid with restrictions  
15-MAR-2004 (597)

**Type:** air  
**Light source:** other: flash lamp  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH  
**Conc. of sens.:** 1000000000000  
**Rate constant:** .00000000000012 cm<sup>3</sup>/(molecule \* sec)  
**Degradation:** day(s)

**Method:** other (measured): determination of the rate constant for the reaction of benzene with OH radicals by a flash photolysis-resonance fluorescence technique  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Result:** Taking a OH radical concentration of 5E+5 radicals/cm<sup>3</sup> a half-life of 13.4 days can be calculated.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Room temperature; flow-through system; initial substance concentration: saturated vapour at -22 - + 22 degree C; total pressure: 133 +/- 3 hPa (argon diluent)

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
15-MAR-2004 (884)

**Type:** air

**Light source:** Sun light

**INDIRECT PHOTOLYSIS**

**Sensitizer:** OH

**Conc. of sens.:** 7500000 molecule/cm<sup>3</sup>

**Rate constant:** = .00000000000013 cm<sup>3</sup>/(molecule \* sec)

**Degradation:** = 50 % after 19 hour(s)

**Method:** other (measured)

**Year:** 1978

**GLP:** no data

**Test substance:** no data

**Result:** The half-life was found to be 19 hours and the residence time 28 hours.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** This study examined the reaction of benzene with OH radicals under photochemical smog conditions in South East England at summer day temperatures. Presumably sunlight was the light source.

**Reliability:** (2) valid with restrictions

15-MAR-2004

(143) (827)

**Type:** air

**Light source:** Sun light

**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**

**Sensitizer:** OH

**Conc. of sens.:** 1100000 molecule/cm<sup>3</sup>

**Rate constant:** = .00000000000013 cm<sup>3</sup>/(molecule \* sec)

**Degradation:** = 50 % after 5.6 day(s)

**Method:** other (calculated)

**Year:** 1979

**GLP:** no data

**Test substance:** no data

**Result:** A half-life for the reaction with OH radicals in ambient air was 5.6 days and the residence time was 8.1 days. In polluted air, with an OH radical concentration of  $1 \times 10^8$  molecules/cm<sup>3</sup>, the half-life was 1.5 hours and the residence time was 2.1 hours.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The half-life was calculated using the method of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. McGraw-Hill Book Company, New York, 1982.

**Reliability:** (4) not assignable

16-MAR-2004

(184) (389) (827)

**Type:** air  
**Light source:** Sun light  
**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**

**Sensitizer:** OH  
**Degradation:** = 50 % after

**Method:** other (calculated)  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Result:** A half-life of 2.4-24 hours was estimated from the reaction rates reported by Davis D.D. et al. J. Phys. Chem. 79, 293-294, 1975 ( $k = 1.59 \times 10^{-12}$  cm<sup>3</sup>/molecule-second) and Hansen D.A. et al. ibid. 79, 1763-1766, 1975 ( $k = 1.24 \times 10^{-12}$  cm<sup>3</sup>/molecule-second). Presumably sunlight was the light source and no indication of sensitizer concentration was given.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (2) valid with restrictions  
15-MAR-2004

(275) (827)

**Type:** air  
**Light source:** Sun light  
**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**

**Sensitizer:** OH  
**Conc. of sens.:** 1200000 molecule/cm<sup>3</sup>  
**Degradation:** = 50 % after 5.3 day(s)

**Method:** other (calculated)  
**GLP:** no data  
**Test substance:** no data

**Result:** A half-life for the reaction between benzene and OH radicals of 5.3 days was calculated using the average Dutch OH radical abundance of  $1.2 \times 10^6$  molecules/cm<sup>3</sup> and the rate constants reported by Davis D.D. et al. J. Phys. Chem. 79, 293-294, 1975 ( $k = 1.59 \times 10^{-12}$  cm<sup>3</sup>/molecule-second), Hansen D.A. et al. ibid. 79, 1763-1766, 1975 ( $k = 1.24 \times 10^{-12}$  cm<sup>3</sup>/molecule-second) and Perry R.A. et al. ibid. 81, 296-304, 1977 ( $k = 1.2 \times 10^{-12}$  cm<sup>3</sup>/molecule-second), measured at 25°C, and those of Tully F.P. et al. ibid. 85, 2262, 1981, Wahner A. & Zetsch C. In: Physico-chemical Behaviour of Atmospheric Pollutants, 138. Proceedings of the 2nd European Symposium, 1981 and Witte F. & Zetsch C. In: Physico-chemical Behaviour of Atmospheric Pollutants, 168. Proceedings of the 3rd European Symposium, Varese. Edited by B. Versino and G.G. Angeletti. D. Reidel Publishing Co., Dordrecht/Boston/Lancaster, 1984 (no rate constant values reported). (All these references were cited in Nielsen et al. 1991). Presumably sunlight was the light source.

Final photochemical reaction products of benzene are glyoxal, butenedial, acroleine, carbonmonoxide and

formaldehyde.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
15-MAR-2004 (827) (1048)

**Type:** air

**Light source:** other: different light sources, see test conditions below

**INDIRECT PHOTOLYSIS**

**Sensitizer:** OH

**Method:** other (measured): different measuring methods, see test conditions below

**Year:** 1983

**GLP:** no data

**Test substance:** no data

**Remark:** Absolute rate constant measurements

**Result:**

Method	t (degree C)	p (hPa)	k (cm <sup>3</sup> /(molecule x s))
A	21	533	(12.2+/-2)E-13
A	23	667	(10.2+/-0.4)E-13
B	22	112	(1.1+/-0.2)E-12
B	25	42	(1.2+/-0.2)E-12
C	23	1030	(9.7+/-0.5)E-13

Note: The above notation of the methods differs from that in the original paper (the method C mentioned above corresponds to method D in the paper).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** A: flash photolysis/resonance fluorescence; light source: spark discharge in N<sub>2</sub> at atmospheric pressure; production of OH radicals by pulsed vacuum UV photolysis of H<sub>2</sub>O (initial OH radical concentration: 5E10 molecules/cm<sup>3</sup>); Ar and He as diluent gas; temperature: 21 and 23 degree C; pressure: 533 and 667 hPa  
B: laser photolysis/resonance fluorescence; light source: excimer laser; production of OH radicals from HNO<sub>3</sub> (initial OH radical concentration: 4E11 molecules/cm<sup>3</sup>); Ar as diluent gas; temperature: 22 and 25 degree C; pressure: 42 and 112 hPa  
C: laser photolysis/laser absorption; light source: excimer laser; production of OH radicals from photolysis of H<sub>2</sub>O at 193 nm or from photolysis of H<sub>2</sub>O<sub>2</sub> at 248 nm (initial OH radical concentration: 1E9 molecules/cm<sup>3</sup>); N<sub>2</sub> or air as diluent gases; temperature: 23 degree C; pressure: 1030 hPa

**Reliability:** (1) valid without restriction

**Flag:** Risk Assessment  
15-MAR-2004 (89)

**Type:** air  
**Light source:** other: different light sources, see test conditions below  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH

**Method:** other (measured): different smog chamber methods, see test conditions below  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Remark:** Relative rate constant measurements  
**Result:**

Method	t (degree C)	p (hPa)	k (cm <sup>3</sup> /(molecule x s))
A	23	267, 933	1.1E-12 (single measurements)
A	27	1000	(1.2+/-0.1)E-12
B	23-32	1050	1.2E-12
C	27	1004	(1.04+/-0.13)E-12

Note: the above notation of the methods differs from that in the original paper:

First mention of A corresponds to E1a,  
 second mention of A corresponds to E2b,  
 B corresponds to F, and  
 C corresponds to G.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** A: chamber volume: 420 and 40 l respectively; light source: photolysis lamps; generation of OH radicals by (a) thermal decomposition of HO<sub>2</sub>NO<sub>2</sub> in the presence of NO and (b) by photolysis of CH<sub>3</sub>ONO; reference substance: n-butane; synthetic air and Ar as diluent gases  
 B: chamber volume: 450 l; light source: UV-A lamps producing photochemical smog in the chamber; NO, NO<sub>2</sub>, O<sub>3</sub> concentrations and relative humidity are monitored; initial concentrations of smog-forming compounds (NO, ethene): ca. 1ppm; initial substance concentrations: ca. 100 ppb; reference substance: toluene; synthetic air as diluent gas  
 C: chamber volume: 520 l; light source: metal halogenide lamp; generation of OH radicals by photolysis of NO<sub>x</sub>; relative humidity: 70 -90%; NO<sub>x</sub> concentration: 300-1000 ppb (with an initial NO/NO<sub>2</sub> ration of 10:1); initial substance concentration: 10-50 ppb; reference substance: ethene; synthetic air as diluent gas

**Reliability:** (1) valid without restriction

**Flag:** Risk Assessment

15-MAR-2004

(89)

**Type:** air  
**Light source:** other: different light sources; compilation of literature data  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH

**Method:** other (measured): different methods; compilation of literature data  
**Year:** 1989  
**GLP:** no data  
**Test substance:** no data

**Result:** At a temperature of 25 degree C the measured rate constants for the reaction of benzene with OH radicals were between  $1.15E-12$  and  $1.59E-12$   $\text{cm}^3/(\text{molecule} \times \text{s})$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
15-MAR-2004 (52)

**Type:** air  
**Light source:** other: flash lamp  
**Light spect.:** > 160 nm  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH  
**Conc. of sens.:** 0 molecule/ $\text{cm}^3$

**Method:** other (measured): vacuum-UV photolysis resonance fluorescence technique  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS: commercial product (purity 99.9%)

**Result:** (a) The reaction rate of the addition of OH radicals to benzene was measured in dependance of the pressure. The rate constants were between  $3E-13$  and  $12.2E-13$   $\text{cm}^3\text{s}^{-1}$  at a temperature of 21 degree C.  
(b) The reaction rate of the addition of OH radicals to benzene was measured in dependance of the temperature. The rate constants were between  $1.11E-12$  and  $1.33E-12$   $\text{cm}^3\text{s}^{-1}$  at a pressure of 200 hPa.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** OH radicals were generated by pulsed vacuum-UV photolysis of  $\text{H}_2\text{O}$  and monitored by resonance fluorescence technique. The test substance was at gas saturation concentration (Ar diluent gas). (a) pressure: 1.3-533 hPa; (b) -34-81 degree C

**Test substance:** The test substance contained 0.15% toluene.  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
15-MAR-2004 (1262)

**Type:** air  
**Light source:** other: flash lamp  
**Light spect.:** > 105 nm  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH

**Method:** other (measured): vacuum-UV photolysis resonance fluorescence technique  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS: commercial product (purity 99.9%)

**Result:** The pressure and temperature dependent measurements of the reaction rate of benzene with OH radicals gave following results:  $k = (0.91-1.04)E-12 \text{ cm}^3\text{s}^{-1}$ . For room temperature at a pressure of 133 hPa an average rate constant of  $(1.02 \pm 0.2)E-12 \text{ cm}^3\text{s}^{-1}$  is given.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** OH radicals were generated by vacuum-UV flash photolysis of H<sub>2</sub>O and monitored by a resonance fluorescence method (radical concentration not given. Benzene was used at gas saturation concentration (He as diluent gas). Pressure range: 33-666 hPa; temperature range: -11--37 degree C.

**Test substance:** The test substance contained 0.04% toluene.  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
15-MAR-2004 (937)

**Type:** air  
**Light source:** other: flash lamp  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH

**Method:** other (measured): flash photolysis-resonance fluorescence technique  
**Year:** 1981  
**GLP:** no data  
**Test substance:** other TS: commercial product (purity > 99.99%)

**Result:** Rate constants  $k$  for the reaction of benzene with OH radicals were  $(1.04-2.20)E-12 \text{ cm}^3\text{molecule}^{-1}\text{s}^{-1}$  in the temperature range between -23 and 744 degree C. At 25 degree C a rate constant  $k$  of  $1.24E-12 \text{ cm}^3\text{molecule}^{-1}\text{s}^{-1}$  was found. In the range between 33 and 267 hPa almost no dependance of the rate constant of the pressure was detected.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Temperature range: -23 - 744 degree C; pressure range: 33-267 hPa; concentration of test substance about 100 times higher than concentration of sensitizer; concentration of sensitizer:  $2E-10 - 1E-11 \text{ molecules/cm}^3$ . The OH radicals were generated by flash photolysis of H<sub>2</sub>O.

**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
15-MAR-2004 (1167)

**Type:** air  
**Light source:** other: super actinic fluorescent lamps  
**Light spect.:** ca. 320 - 480 nm  
**Conc. of subst.:** at 27 degree C

**DIRECT PHOTOLYSIS**

**Halflife t1/2:** > 160 minute(s)

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other: Bromine atoms  
**Conc. of sens.:** 10000000000 molecule/cm<sup>3</sup>  
**Rate constant:** < .000000000000000003 cm<sup>3</sup>/(molecule \* sec)

**Method:** other (measured): relative kinetic method  
**Year:** 1998  
**GLP:** no data  
**Test substance:** other TS

**Method:** A relative kinetic method using the photolysis of Br<sub>2</sub>-molecules as a source of Br-atoms was employed. Analytical monitoring over a time of 15 - 60 min was performed by capillary-GC-FID. As reference compound, ethanol with a reaction rate constant of  $9.2 \times 10^{(-15)}$  was employed.

A control experiment showed that the photolysis frequency as well as wall loss reactions and dark reactions with bromine molecules were negligible.

**Result:** The reaction of benzene with Br atoms was negligible:  $k \leq 5 \times 10^{(-16)}$  with a life time above 1.3 years.

**Source:** German Rapporteur

**Test condition:** 20 l and 400 l Duran glass reaction chamber surrounded by 4 and 18 super actinic fluorescent light resp. (Philips TL 05/40 W: 320 - 480 nm, maximum at 360 nm) at 1013 hPa synthetic air and  $300 \pm 2$  K.  
The Br-atom concentration was  $10^{*9}$  to  $10^{*10}$  Br/cm<sup>3</sup>. The benzene concentration was  $(3 - 6) \times 10^{*14}$  molecules/cm<sup>3</sup>.

**Test substance:** > 97 % (Aldrich)

**Reliability:** (2) valid with restrictions

15-MAR-2004

(101)

**Type:** air  
**Light source:** Sun light  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other: Cl atoms

**Method:** other (measured): monitoring program  
**Year:** 1999  
**GLP:** no data  
**Test substance:** no data

**Method:** Grab sampling of air 1 - 6 times a day on the Julian days 57 - 113, 1995 in canisters. Analysis by capillary-GC-FID with cryogenic trapping, identification by reference gas mixtures, quantification by calibration with external standards.

**Result:** Distinction of reactions with OH radicals and Cl atoms was achieved by following the ratios of 2-methylpropane/n-butane (constant for OH kinetics, increasing with time for Cl oxidation) and 2-methylpropane/propane (decreasing with time

for OH kinetics, remaining constant for Cl oxidation). Oxidation by Cl atoms coincides with low ozone concentrations after polar sunrise. Non-methane hydrocarbons occur in different concentrations with normal ozone concentrations (background levels b<sub>gk</sub>) and at low ozone concentrations (low). The difference between both concentrations can be attributed to reactions with Cl atoms, so from these data, the Cl atom concentration (as a lower limit) can be deduced. During ozone depletion, benzene reacted with Cl atoms the concentration of which was determined as mentioned above. The reaction rate constant of benzene with Cl atoms is  $10^{12}$  cm<sup>3</sup>/molecule/s.

**Source:** German Rapporteur

**Test condition:** Monitoring of hydrocarbons and their variation with time before, during and after polar sunrise in the Northwest Territories, Canada.

**Reliability:** (2) valid with restrictions  
15-MAR-2004

(42)

**Type:** air  
**Light source:** Xenon lamp

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other: NO  
**Conc. of sens.:** 1.2 mg/l

**Method:** other (measured): batch test in closed quartz vessel  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Remark:** Levels of degradation products not given  
**Result:** Degradation to nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol was found. During the photochemical reaction the initial substance concentration decreased by about 10 %. About 16 % of the decreased benzene was converted into nitrobenzene (5.5%), nitrophenols (7.7%) and dinitrophenols (2.4 %).

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Benzene (6.32 mg/l) was tested in an air-NO system in a 1 l quartz reaction vessel (concentration of substance converted from cited 2 ml in 1 litre reaction vessel implies concentration was 0.2% v/v = 6.32 mg/l of benzene; concentration of sensitizer converted from cited 1 ml in 1 litre reaction vessel implies concentration was 0.1% v/v = 1.2 mg/l of NO). Irradiation was conducted for 5 h at 25-30 degree C and degradation followed by thin-layer chromatography, gas chromatography and gas chromatography-mass spectrometry.

**Reliability:** (2) valid with restrictions  
15-MAR-2004

(836)

**Type:** air  
**Light source:** other: black light lamps  
**Light spect.:** 300 - 430 nm  
**Conc. of subst.:** .139 mg/l at 22.5 degree C  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other: NO2

**Method:** other (measured): determination of photodegradation products after irradiation in FTIR cell  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS: commercial product (reagent grade; purity not given)

**Remark:** Very high concentrations of test substance  
**Result:** Main photoproducts of the irradiation of a benzene/NO2 mixture were phenol (ca. 0.16 ppm), nitrobenzene (ca. 0.8 ppm), glyoxal (ca. 0.05 ppm), formaldehyde (ca. 0.03 ppm), formic acid and maleic anhydride (ca. 0.01 ppm each) (concentrations given after an irradiation time of 180 min). It is suggested that phenol and nitrobenzene are both generated from the OH-benzene adduct while glyoxal is a ringcleavage product.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Substance concentration: 42.65 ppm; concentration of sensitizer: 1.50 ppm; pressure: 1013 hPa; the chemical structure and the concentration of the photoproducts was determined by FTIR.

**Reliability:** (2) valid with restrictions  
15-MAR-2004 (68)

**Type:** air  
**Light source:** other: blacklight fluorescent lamps  
**Light spect.:** 345 - 355 nm  
**Conc. of subst.:** .006 mg/l at 30 degree C  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other: NOx  
**Degradation:** = 16.5 % after 5 hour(s)

**Method:** other (measured): smog chamber test  
**GLP:** no data  
**Test substance:** no data

**Remark:** In another study, benzene was reported to be unreactive in air, based on the maximum ozone concentration produced (average 3.1% degradation/hour) when 4 ppm benzene (0.013 mg/l) was irradiated in a pyrex glass chamber with 22 black lights and 7 sunlamps in the presence of 0.2 ppm NOx (Dimitriades B. & Joshi S.-B. In: EPA-600/3-77-001B. Inter. Conf. on Photochemical Oxidant Pollution and its Control. p.705-711. Edited by B. Dimitriades. US Environmental Protection Agency, Research Triangle Park, North Carolina, 1977). A similar study in which a benzene-NOx-air system was irradiated in a pyrex flask, showed 2% of the degradation reaction occurring in the first hour and carbon dioxide, carbon monoxide, formic acid, peroxyacetyl nitrate and an unspecified aldehyde to be formed as the degradation

products (Kopczynski S.L. Int. J. Air Wat. Pollut. 8, 107-120, 1964). Finally, when 100 ppm benzene (0.32 mg/l) was irradiated, in the presence of 10-110 ppm NOx, with light of wavelength >290 nm, photodegradation occurred with a half-life of 4-5 hours. Approximately 2 days were required for 50% of the benzene to degrade to carbon dioxide (Korte F. & Klein W. Ecotoxic. envir. Saf. 6, 311-327, 1982) (all cited in Envirofate, 1993).

**Result:** The intensity of irradiation corresponded to K1 = 0.33/min of nitrogen dioxide (NO2) photo-dissociation rate, and 16.5% depletion of benzene was seen in 5 hours. The depletion rate for nitric oxide was found to be 2.2 ppb/min and a maximum concentration of 0.34 ppm NO2 was seen after 300 minutes. No ozone formation was recorded and, after 2 and 4 hours, formaldehyde was measured at 0.02 and 0.03 ppm respectively. NOx was decreased to 0.3 ppm after 5 hours.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The experiment was conducted with the Mechanical Engineering Laboratory Smog Chamber. The light source was 300 UV fluorescent lamps of "Black light" of relative intensity 700 µW/cm<sup>2</sup>. Nitrogen oxides were used as the sensitizer at a concentration of 1 ppm (conversion impossible). Substance concentration cited as 2 ppm (converts to 0.006 mg/l). The relative humidity was controlled at 55%. Irradiation was continued for 5 hours. NO2 was measured using the Saltzman method or UV spectro-photometer and nitric oxide was measured by the chemiluminescence method. NOx was reduced to NO by a converter, for measurement. Ozone was measured by the chemiluminescence of the reaction with ethylene and formaldehyde by the para-rozani-line method. Benzene was analysed with a gas chromatograph.

**Reliability:** (2) valid with restrictions

15-MAR-2004

(184) (1291)

**Type:** air

**Light source:** other: light with wavelength corresponding to tropospheric sunlight

**Light spect.:** > 290 nm

**Conc. of subst.:** .32 mg/l

**Method:** other (measured)

**Year:** 1982

**GLP:** no data

**Test substance:** no data

**Remark:** Documentation insufficient for assessment

**Result:** No evidence of benzene degradation was found.

After the addition of chemicals which produce active species benzene half-lives were between 4 and 5 h:

100 ppm benzene + 10 - 100 ppm nitrogen oxides: 6.5 h

100 ppm benzene + 10 - 100 ppm sulphur dioxide: 5 h

100 ppm benzene + 10 - 100 ppm nitrogen oxides

+ 5 ppm isobutane: 4 h

**Source:** It is noted that ambient air is rarely clean or dry.  
BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Initial substance concentration: 100 ppm (=0.32 mg/l).  
Benzene exposed in a 20 l smog chamber filled with clean,  
dry air. Irradiation with light at a wavelength  
corresponding to tropospheric sunlight occurred for 6 days.  
No further details were available.

**Reliability:** (4) not assignable  
15-MAR-2004 (184) (634) (827)

**Type:** air

**Light source:** other: mercury vapour lamp with a water-cooled quartz filter

**Light spect.:** > 230 nm

**Conc. of subst.:** .32 mg/l at 25 degree C

**DIRECT PHOTOLYSIS**

**Degradation:** = 31.5 % after 2 hour(s)

**Method:** other (measured)

**Year:** 1981

**GLP:** no data

**Test substance:** no data

**Result:** Cited value of 100 ppm converted to 0.32 mg/l.  
68 ppm carbon dioxide and 121 ppm carbon monoxide (convert  
to 0.12 and 0.14 mg/l respectively) were detected. The  
half-life was not reported.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Benzene exposed in a 4.4 l reactor for 2 hours. Carbon  
dioxide and carbon monoxide degradation products were  
measured by infrared-gas chromatography in the case of  
carbon dioxide or gas chromatography with a molecular sieve  
column ultrasound detector in the case of carbon monoxide.

**Reliability:** (4) not assignable  
16-MAR-2004 (542)

**Type:** air

**Light source:** other: low pressure mercury lamp

**Light spect.:** 254 nm

**Quantum yield:** .05

**Method:** other (measured): test in a free flow system

**Year:** 1993

**GLP:** no data

**Test substance:** other TS: commercial product (purity 99.5%)

**Result:** standard deviation: +/- 0.02  
quantum yield (185 nm): 0.01 +/- 0.005

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** wavelength 254 nm: substance concentrations 0.13, 1.3 and 8  
hPa; NiSO4-filter to eliminate ca. 99% of the 185 nm  
radiation from the lamp  
wavelength 185 nm: substance concentrations 0.1 and 1.3

hPa;O3-filter to eliminate > 99% of the 254 nm radiation from the lamp and of the ethene photolysis as an actinometer reaction

**Reliability:** (1) valid without restriction  
15-MAR-2004 (597)

**Type:** air  
**Light source:** Sun light  
**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other  
**Conc. of sens.:** 72000 molecule/cm<sup>3</sup>  
**Rate constant:** = .0000000000000028 cm<sup>3</sup>/(molecule \* sec)  
**Degradation:** = 50 % after 10.9 year

**Method:** other (calculated)  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Result:** The half-life for the reaction between benzene and atomic oxygen presumably in sunlight was calculated to be 10.9 years. The formation of phenol has been reported as a result of such a reaction (Altshuller A.P. & Bufalini J.J. *Envir. Sci. Technol.* 5, 39, 1971; ATSDR, 1987). This is not reported in the 1991 ATSDR draft report (ATSDR. Draft Toxicological Profile for Benzene. Agency for Toxic Substances and Disease Registry, Public Health Service, US Department of Health and Human Services, 1991).

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The half-life for the reaction between benzene and atomic oxygen, presumably in sunlight, was calculated using the method of Lyman W.J. et al. *Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds*. McGraw-Hill Book Company, New York, 1982 and the reaction rate constant of Gaffney J.S. & Levine S.Z. *Int. J. Chem. Kinet.* 11, 1197-1209, 1979 (cited in *Envirofate*, 1993; Nielsen et al. 1991).

**Reliability:** (4) not assignable  
16-MAR-2004 (53) (331) (827)

**Type:** air  
**Light source:** Sun light  
**Conc. of subst.:** .016 mg/l at 37 degree C

**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other  
**Conc. of sens.:** .004 mg/l

**Method:** other (measured)  
**Year:** 1963  
**GLP:** no data

**Remark:** A second study conducted in a smog chamber with 1-10 ppm benzene (0.003-0.032 mg/l) and 0.1-3 ppm NO (0.1 x 10<sup>-3</sup> - 3.7 x 10<sup>-3</sup> mg/l) reported a low reactivity with the maximum NO<sub>2</sub> formation occurring after 290 minutes (Levy A. *Adv.*

Chem. Ser. 124, 70-94. Am. Chem. Soc. Washington D.C., 1973 (cited in Envirofate, 1993).

**Result:** The time for the NO<sub>2</sub> formation to reach a maximum concentration was 210 minutes in the FEP bag. No details of the Mylar bag were given.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** A Mylar or FEP bag smog chamber was used. The light source was artificial sunlight (warm white, sun and blacklight fluorescent lamps) and the sensitizer was NO. The FEP bag passes 30-40% more radiation in the 330-400 nm region and much more less than 330 nm than the Mylar bag. The colorim method of analysis was used.

**Reliability:** (4) not assignable  
16-MAR-2004 (23) (184)

**Type:** air  
**Light source:** other  
**Conc. of subst.:** .003 mg/l at 26 degree C

**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other

**Method:** other (measured)  
**Year:** 1970  
**GLP:** no data  
**Test substance:** no data

**Remark:** In a second study, benzene was tested at 0.003 mg/l in a smog chamber in the presence of 0.45 ppm NO and 0.05 ppm NO<sub>2</sub> (converts to  $0.6 \times 10^{-3}$  and  $0.9 \times 10^{-4}$  mg/l). The rate of photolysis was found to be 1.4 ppb/min (converts to 4.5 µg/l/min) (Dimitriades B. et al. Development and Utility of Reactivity Scales from Smog Chamber Data. RI 8023. US Bureau of Mines, Pittsburgh, Philadelphia, 1975 (cited in Envirofate, 1993).

**Result:** The rate of photolysis was 0.33 ppb/minute (converts to 1.05 ng/l/min). The rate constant (radical),  $k(\text{NO}_2)$ , was 0.29 l/min.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** Concentration of substance 0.003 mg/l converted from cited 1 ppm. Concentration of sensitizer (NO)  $0.5 \times 10^{-3}$  mg/l converted from cited 0.38 ppm. Concentration of sensitizer (NO<sub>2</sub>)  $0.4 \times 10^{-4}$  mg/l converted from cited 0.02 ppm. The test was conducted in a simulated atmosphere using black fluorescent lamps. The sensitizer was NO and NO<sub>2</sub>.

**Reliability:** (2) valid with restrictions  
15-MAR-2004 (184) (415)

**Type:** air  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other

**Method:** other (measured)  
**GLP:** no data  
**Test substance:** no data

**Result:** The degradation products were found to be phenol and nitrobenzene.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** Benzene was photolysed in air in the presence of NO, NO2 and HNO2. No further details were available.

**Reliability:** (4) not assignable  
15-MAR-2004 (184) (524)

**Type:** air  
**Light source:** Sun light  
**Light spect.:** > 290 nm  
**Conc. of subst.:** .32 mg/l  
**INDIRECT PHOTOLYSIS**  
**Sensitizer:** other  
**Conc. of sens.:** .012 mg/l  
**Degradation:** = 50 % after 16 hour(s)

**Method:** other (measured)  
**GLP:** no data  
**Test substance:** no data

**Result:** Photodegradation seen, with approximately 2 days required for 50% degradation to carbon dioxide. Half-life found to be 16 hours.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** No further information available on method. The sensitizer was isobutane at a concentration cited as 5 ppm (converts to 0.012 mg/l). The light source was light at a wavelength corresponding to tropospheric sunlight.

**Reliability:** (4) not assignable  
15-MAR-2004 (184) (634)

**Type:** other: test substance adsorbed onto different solid surfaces  
**Light source:** other: different irradiation conditions including sun light

**Method:** other (measured): photomineralization tests with test substance adsorbed onto silica gel and natural sand respectively

**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Result:**

Test medium	Irradiation time (d)	Light source	Photomineralization (% CO2)
silica gel	6 d	> 290 nm	12-73
natural sand	30 d	sunlight	< 1

Formation of CO<sub>2</sub> was dependent on water content of the solid. There was an initial buildup of a high amount of intermediates with significantly delayed CO<sub>2</sub> formation.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** No further details were available.

**Reliability:** (4) not assignable  
15-MAR-2004 (634)

**Type:** water  
**Light source:** Sun light

**INDIRECT PHOTOLYSIS**  
**Sensitizer:** OH  
**Degradation:** = 50 % after 80 day(s)

**Method:** other (measured)  
**GLP:** no data  
**Test substance:** no data

**Result:** A half-life of 80 days has been reported for the reaction taking place just below the water surface between benzene and OH radicals. The half-life will tend to increase with depth as a result of both decreased light intensity and OH radical concentration.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** No further details on test method or conditions available.

**Reliability:** (4) not assignable  
16-MAR-2004 (827) (1048)

**Type:** water  
**Light source:** Sun light

**DIRECT PHOTOLYSIS**  
**Halflife t<sub>1/2</sub>:** 8.8 - 673 day(s)

**Method:** other (measured): according to Leifer, A. & Stern, A.M., Test guideline: Photochemical transformation in water. US EPA, Washington, date not specified  
**GLP:** no data  
**Test substance:** no data

**Remark:** Experiments were carried out in five different laboratories. The authors state that the broad range of values obtained may have been due to loss due to high volatility, varying intensity of sunlight or unsuitable methods of analysis. In any case the relevance of these findings and methodology must be limited by the predominance of volatilization as a removal process for benzene in aquatic systems.

**Result:** Degradation was reported but no indication of extent was given.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of Leifer A. & Stern A.M. Test guideline: Photochemical transformation in water, EPA, Washington, date unspecified, was used. Experiments were carried out in five different laboratories.

Benzene at a concentration of < 900 mg/l was exposed to sunlight in a closed vessel of duran glas. The substance loss was determined gaschromatographically in the water phase.  
No further details were available.

**Reliability:** (3) invalid  
16-MAR-2004 (543)

**Type:** water  
**Light source:** Sun light  
**DIRECT PHOTOLYSIS**  
**Halflife t1/2:** 8.8 - 673 day(s)

**Method:** other (measured): according to Leifer, A. & Stern, A.M., Test guideline: Photochemical transformation in water. US EPA, Washington, date not specified  
**GLP:** no data  
**Test substance:** no data

**Remark:** Experiments were carried out in five different laboratories. The authors state that the broad range of values obtained may have been due to loss due to high volatility, varying intensity of sunlight or unsuitable methods of analysis. In any case the relevance of these findings and methodology must be limited by the predominance of volatilisation as a removal process for benzene in aquatic systems.  
significant methodological deficiencies

**Result:** Degradation was reported but no indication of extent was given.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of Leifer A. & Stern A.M. Test guideline: Photochemical transformation in water, EPA, Washington, data unspecified, was used. Experiments were carried out in five different laboratories.  
Benzene at a concentration of < 900 mg/l was exposed to sunlight in a closed vessel of duran glas. The substance loss was determined gaschromatographically in the water phase.  
No further details were available.

**Reliability:** (3) invalid  
15-MAR-2004 (543)

**Type:** water  
**Light source:** Sun light  
**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other  
  
**GLP:** no data  
**Test substance:** no data

**Result:** Reaction with singlet oxygen is negligible due to low reactivity, the rate constant having been reported at  $<<360/M\text{-hour}$ . This is similarly true in the presence of alkyl peroxy radicals ( $RO_2$ ), with the rate constant being reported as  $<<1/M\text{-hour}$ . In any case the relevance of these findings and methodology must be limited by the predominance of volatilization as a removal process for benzene in aquatic systems.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004

(332) (827)

**Type:** water  
**Light source:** Sun light  
**Conc. of subst.:** 900 mg/l

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other  
**Degradation:** = 50 % after 16.9 day(s)

**Method:** other (measured)  
**GLP:** no data  
**Test substance:** no data

**Remark:** The relevance of these findings are limited by the predominance of volatilization as a removal process for benzene in aquatic systems.

**Result:** The rate constant (radical) for the reaction was 0.041/day.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The photolysis of benzene at a concentration of  $<900\text{ mg/l}$  dissolved in deionized water saturated with air and exposed to sunlight was measured. Benzene concentrations were measured by gas chromatography with flame-ionization detection.

**Reliability:** (4) not assignable  
16-MAR-2004

(543)

**Type:** water  
**Light source:** Sun light  
**Conc. of subst.:** at 25 degree C

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other  
  
**GLP:** no data  
**Test substance:** no data

**Result:** Reaction with singlet oxygen is negligible due to low reactivity, the rate constant was reported at <<360/M-hour. This is similarly true in the presence of alkyl peroxy radicals (RO<sub>2</sub>), with the rate constant being reported as <<1/M-hour. In any case the relevance of these findings and methodology is limited by the predominance of volatilisation as a removal process for benzene in aquatic systems.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable

15-MAR-2004

(333) (826)

**Type:** water  
**Light source:** Sun light  
**Conc. of subst.:** 900 mg/l

**INDIRECT PHOTOLYSIS**

**Sensitizer:** other  
**Degradation:** 50 % after 16.9 day(s)

**Method:** other (measured)

**GLP:** no data  
**Test substance:** no data

**Remark:** The relevance of these findings is limited by the predominance of volatilisation as a removal process for benzene in aquatic systems.

**Result:** The rate constant (radical) for the reaction was 0.041/day.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The photolysis of benzene at a concentration of < 900 mg/l dissolved in deionized water saturated with air and exposed to sunlight was measured. Benzene concentrations were measured by gas chromatography with flame ionisation detection.

**Reliability:** (4) not assignable

15-MAR-2004

(543)

**Type:** other  
**Light source:** other: not specified

**GLP:** no data  
**Test substance:** no data

**Remark:** Direct photolysis of benzene is not expected due to low absorbance of UV light.  
Reliability not assignable; quotation from another paper^

**Source:** Used for Risk Assessment.  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (4) not assignable  
14-OCT-2002 (161) (528)

**Type:** other  
**Light source:** other: not specified

**GLP:** no data  
**Test substance:** no data

**Remark:** Direct photolysis of benzene is not expected due to low absorbance of UV light.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (4) not assignable  
**Flag:** Risk Assessment  
18-MAR-2004 (528)

### 3.1.2 Stability in Water

**Type:** abiotic

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Result:** Hydrolysis is not a significant process for benzene and it will not be expected to significantly adsorb to sediment.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (527)

**Type:** abiotic

**Method:** other: estimation

**Result:** Benzenes appear to be generally resistant to hydrolysis.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (4) not assignable  
**Flag:** Risk Assessment  
16-MAR-2004 (483)

**Type:** abiotic  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Hydrolysis of benzene at typical environmental conditions is not likely due to the lack of reactive functional groups in the molecule.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (4) not assignable  
**Flag:** Risk Assessment  
16-MAR-2004 (529)

### 3.1.3 Stability in Soil

**Type:** laboratory  
**Radiolabel:** no  
**Concentration:** 39.055 ppm  
**Soil temperature:** 30 degree C  
**Soil humidity:** 60 g water/100g soil dry weight  
**pH:** = 7

**Method:** other  
**Year:** 1989  
**GLP:** no data  
**Test substance:** no data

**Result:** The indigenous microflora needed about 30 hours for adaptation to degrade benzene and 100 hours for the complete removal of benzene.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** Unsterilized soil slurries were prepared from garden soil (clay) in MMY-medium. After addition of 500 µM benzene, the soil was stored for 2 months at 4°C to allow adsorption to take place. A gas-tight system was used for incubation, to prevent volatilization. The flasks were rotated during incubation to ensure mixing. Degradation was measured by gas chromatography.

**Test substance:** Test substance was laboratory reagent grade.  
**Reliability:** (2) valid with restrictions  
15-MAR-2004 (857)

**Type:** laboratory  
**Radiolabel:** yes  
**Concentration:** 20 mg/kg  
**Soil humidity:** 7 g water/100g soil dry weight  
**Organ. carbon:** 1.3 %  
**pH:** 7.1

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Result:** The levels of carbon dioxide released were found to be 7.5, 24, 37, 44 and 47% after 3 days and 1, 2, 5 and 10 weeks respectively, showing degradation.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** A base-rich, para-brownish, loess (clay) soil from Flachstoeckheim near Braunschweig/FRG (100 g; 0.12% nitrogen) was mixed with 2 mg <sup>14</sup>C-labelled benzene dissolved in 0.1 ml acetone, and moistened to 70% of the water capacity. [10 ml water; 70% of water capacity (10 ml water in 100 g soil implies 7% w/w)]. <sup>14</sup>CO<sub>2</sub> production was measured over a period of 10 weeks.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
15-MAR-2004

(466)

**Type:** other  
**Organ. carbon:** = 1.3 %

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** If released to soil, benzene will be subject to rapid volatilization near the surface. That which does not evaporate will be highly to very highly mobile in soil and may leach to groundwater. The effective half-lives for volatilization from soil, without water evaporation, of benzene uniformly distributed to 1 and 10 cm in soil with an organic carbon content of 1.25%, were 7.2 and 38.4 days respectively.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
15-MAR-2004

(331) (589)

**3.2.1 Monitoring Data (Environment)****Type of measurement:** other: emissions into air, concentrations and degradation in air.**Medium:** air**Method:** GC-FID/MS**Remark:** General Comments: Air monitoring of 66 C2-C10 hydrocarbons from distinct, different locations in the city of Porto Alegre, Brazil. Sampling frequency and location: urban (U) and background (B) stations: total of 44 (U) samples collected year long every one or two weeks from downtown monitoring station located on divider strip of major highway, not far from major terminal for buses and trucks; two (B) samples from station approx. 30 km SE of city and upwind from city during sample coll'n. Measured conc reported, additionally calculated emission rates/yr, and reactivity ranking for OH rx and O3 production (1 = ethylene w/highest rx , 66 = 2,3-dimethylhexane w/lowest rx). Both m/p-xylenes and toluene are significant atmospheric emissions due to estimated releases and reactivity ranking.**Result:** Measured Benzene Conc. - mg/m3 (SD)  
Urban samples 11.8 (6.9)

Background samples 0.4 (0.8)

Vehicle Emissions 907  
(tn/yr) - calc (229)Reactivity Ranking 51  
Rx w/OHReactivity Ranking 45  
O3**Source:** Exxon Biomedical Sciences, Inc. East Millstone, NJ USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

16-MAR-2004

(451)

**Type of measurement:** concentration at contaminated site**Medium:** other: air in city centers**Method:** GC**Remark:** General Comments: Air monitoring of aromatic hydrocarbons (plus addt'l analytes) at two stations in areas of high traffic in the city of Rieti, Italy. Sampling frequency: station 1(S1): 5 hr sampling interval performed daily for one week in March, Aug, & Nov; station 2 (S2): sample frequency performed in Aug. & Nov. Results reported as ng/m3 and indicate seasonal trend; conc increase at both stations in Nov and Dec concluded to be due to "unfavorable climatic conditions (no clarification) and an increase in traffic volume (no supporting data). Although no statistics are presented for monthly analysis, this trend is supported by similar results for other analytes (PAHs). In general, benzene conc fall below limits of Italian

national ministerial decree (D.M.)25-11-94, during annual monitoring events. Seasonal fluctuations in conc for BTEX were also observed and theorized to be due to traffic patterns and temperature cooler air preventing extensive volatilization of BTEX.

**Result:** Benzene Concentration - ng/m3

Month of Sample		November	December
April	August	9.8	8.5
5.8	3.0; 3.2		
(S1)	(S1);		

**Source:** Exxon Biomedical Sciences, Inc. East Millstone, NJ, USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

16-MAR-2004

(456)

**Type of measurement:** background concentration

**Medium:** biota

**Method:** Analysis by Purge/Trap coupled to GC-FID, MS

**Remark:** General Comments: A method was developed that allows the simultaneous determination of the volatile organochlorines (VOCs and the volatile aromatics benzene, toluene, ethylbenzene and the xylenes (BTEX) in homogenised biological tissue from marine biota using purge-and-trap apparatus coupled to a gas chromatography-mass spectrometry (GC-MS) system. Identification and quantification were performed with mass spectrometry operated in the electron impact mode. The method allows detection limits between 0.005 ng/g and 0.2 ng/g depending on the background levels and the amount of sample. The method was used to determine the concentrations of VOCs in *Limanda limanda* (dab) and *Merlangius merlangus* (whiting) collected at two sampling stations located on the Belgian continental shelf. Liver and muscle tissue were individually analysed in order to determine the inter-species and inter-specimen variability.

**Result:** Benzene concentrations (mean ng/g) in fish sampled from the Belgian continental shelf

Fish Species	<i>Merlangius merlangus</i> (whiting)		<i>Limanda limanda</i> (dab)	
	Liver	Muscle	Liver	Muscle
Tissue Type	5.96	4	11.8	0.54

The results show a considerable variability within tissues of the same species (R.S.D., 50-200%). In most cases, the concentrations of the VOCs appeared to be normally distributed. These results are similar to the limited literature reporting quantitative data, and reported water concentrations are an order of magnitude lower for BTEX, ranging between 10 - 50 ng/L as compared to ng/g concentrations in fish tissue. Although the concentrations in fish tissue are somewhat higher than water concentrations, biomass concentrations are generally low, as expected, as the BCF values and log P values are on the same order, indicating limited potential to bioaccumulate and bioconcentrate.

**Source:** Exxon Biomedical Sciences, Inc. East Millstone, NJ, USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

16-MAR-2004

(956)

**Type of measurement:** other: exchange between air masses and sea water

**Medium:** other: air and water

**Method:** GC-FID

**Remark:** During six campaigns in September 1994-December 1995, simultaneous air and water sampling was carried out in both the southern North Sea area and the Scheldt Estuary (Belgium region). Analytical results and partitioning estimates for benzene, toluene, ethylbenzene and xylenes are summarized in at the end of this review. Mean water concentrations are consistent with those data cited in the literature, with the exception of (m, p) xylenes, of which the mean sample data was slightly higher (69 ppt) compared to the concentration range of 1-42 ppt found in the literature. Calculations showed that a number of over sea atmospheric samples were affected by atmospheric transport from industrial sites, 250-300 km upwind. Air samples of continental origin (wind direction from European continent during sampling). Air samples of continental origin (wind direction from European continent during sampling) had significantly higher benzene and toluene concentrations ( $\alpha = 0.05$ ) as compared to air samples of marine origin (wind direction from ocean during sampling). Air mass from highly industrial areas (sites in Nantes, Rennes, Caen, and Le Havre, France; and the Ruhr area, Germany) were identified as contributing to these air sample with continental origin. Based on analytical data, transport/equilibrium predictions for air to and water to air exchange rates (flux) indicate that the North Sea may serve as a source of VOCs to the atmosphere. However, this contribution may be limited when continental air masses are present. It was statistically shown that water to air exchange rates and toluene were slowed when air masses from continental origin were at the marine sampling sites instead of air masses from remote noncontinental origin.

**Result:** Benzene Concentrations in Air and Water Samples from the North Sea and Scheldt Estuary; Air/Water Exchange

Mean Measured Concentration (range)

Water-ng/L 15 (13-19)

Atmosphere-pptv 413 (182-479)

Air Concentration Range (pptv)

Continental Air Mass 200 - 700

Ocean Air Mass 200 - 300

Air/Water Fugacity  
Ratio( $f_a/f_w$ ); R values 0.80

Water to Air Exchange      3.9  
 Rate(flux, ug/m2/d)

The air to water exchange R values >1 indicate deposition, values <1 indicate volatilization.

**Source:** Exxon Biomedical Sciences, Inc. East Millstone, NJ, USA  
 Exxon Chemical Europe Inc. Bruxelles  
 EXXON Biomedical Sciences East Millstone, NJ  
 German Rapporteur

16-MAR-2004

(296)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Remark:** Rapid in-situ quasi-continuous GC/FID measurements of BTEX were carried out at 2 urban sites (500-1000 samples/site) in Munich/Germany for 7-10 days (10-11/93). Site 1 (monitoring industrial emissions): IHF = close to Institut fur Holzforschung, and in vicinity of industrial area (BMW factory). Site 2 (traffic emissions): UMW= in vicinity of main railway station near Umweltschutzreferat der Landeshauptstadt (Env. Protection Dept). A cycle time of 10 min. allowed detn. of short-time BTEX emission variations and their impact on selected BTEX ratios.

**Result:** Results indicate that higher levels of BTEX emissions occur at IHF (industrial area). Benzene/toluene ratios (B/T, traffic emissions ratios), were w/in the average worldwide value range for urban areas. Low values for this ratio indicate toluene emissions originate from both traffic and solvents use. Similarly, lower B/X ratios indicate higher xylene emissions from solvent use, and based on meas data, toluene and xylene isomer emissions at IHF have a major common source, organic solvent use. Ethylbenzene/m+p-xylene (E/X) ratio showed the smallest variations even in case of rapid changes in BTEX, relatively independent of emission strength. This ratio is suggested to be primarily dependent on photochem. processes in urban environments, as the ratio will increase during periods of high photochemical reactivity (ie, summer daylight enhanced OH radical attack) since m/p-xylene have 2-4 times faster reaction OH rates than ethylbenzene. The E/X emission ratio can serve as an indicator for the impact of urban hydrocarbon emissions leading to the formation of secondary pollutants.

Benzene (ppbv) at IHF and UMW in metropolitan Munich					
IHF			UMW		
Mean	Median	Max	Mean	Median	Max
3.5	2.3	18.7	2.9	2.7	8.2

BTEX-Ratio	IHF			UMW		
	Mean	Range	SD%	Mean	Range	SD%
B/T	0.56	0.3-1.11	12	0.77	0.45-1.84	18
T/X	2.41	0.2-8.26	27	2.43	1.9-3.99	13
B/X	1.39	0.22-4.13	21	1.87	1.17-6.64	29
E/X	0.34	0.25-1.06	15	0.36	0.3-1.06	15

**Source:** Exxon Biomedical Sciences, East Millstone, NJ, USA  
 Exxon Chemical Europe Inc. Bruxelles

EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

16-MAR-2004

(924)

**Type of measurement:** background concentration**Medium:** air

**Remark:** Air samples were collected once a week at urban (n = 34 samples) and suburban (n = 44 samples) sites in Martorell, Spain (10/1992 to 7/1993). Metrological data was recorded continuously at each half hour, air sampling time varied 5-24 hrs. Samples analyzed for VOCs using GC/MS. Airborne concns. of several VOCs were measured in the city of Martorell (Spain) and compared to VOC levels in 16 other cities of the world. Mean concentrations were found to be higher at the urban site as compared to the suburban site. Concentrations during the summer were lower (factor of 2) at the urban site compared to data obtained during winter seasons, in part due to photochemical reactivity increases promoted by increased solar radiation. However, suburban VOC concentrations during summer and winter months were comparable, probably due to higher summer traffic emissions due to an increase in vacation travel on the major roadways in this region, combined with similar photochemical depletion of VOCs as was concluded for urban area concentrations. In comparison to data from other cities, air concentrations for BTEX are w/in the range of values reported.

**Result:** Mean Benzene Concentrations (ug/m3 +/- SD):

Urban Area	Suburban Area	Urban Range (16 cities)
3.8 (3.5)	1.3 (1.3)	31 - 3.5

**Source:** Exxon Biomedical Sciences Inc., East Millstone, NJ, USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

16-MAR-2004

(67)

**Type of measurement:** background concentration**Medium:** air

**Result:** In the United Kingdom, urban atmospheric levels have been reported to range from 0.01 ppb (approximately 32 ng/m3) in a poorly ventilated traffic tunnel in London, to 576 µg/m3 measured during a 1973 study of various sites around London. The majority of the benzene concentrations reported are below 100 µg/m3.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(530) (827) (883) (1162)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In the United Kingdom, rural atmospheric concentrations have been reported to range from 1.3 µg/m<sup>3</sup> (arithmetic mean hourly concentration measured in 1983 in Silwood Park about 1 km for the nearest main road) to 292 µg/m<sup>3</sup> (mean daily concentration from at least seven 24-hour samples taken in Heston, Middlesex under the Heathrow airport flight-path). The majority of the concentrations reported are below 20 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(215) (827) (1128)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In the United Kingdom, atmospheric benzene concentrations at sites near motorways have been reported to range from 0.2 µg/m<sup>3</sup> (arithmetic mean hourly concentrations measured at Toddington, about 15 metres from the M1 Motorway in 1983) to 489 µg/m<sup>3</sup> (mean daily concentrations from at least seven 24-hour samples taken from a garden in Luton about 150 yards from the motorway). All such reported benzene concentrations, apart from the latter, are below 45 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(215) (827) (1128)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In April 1986-1987, several sites around Hamburg, West Germany, were assessed for their atmospheric benzene concentrations. In a populated urban site with dense traffic, benzene was present at 15.3 µg/m<sup>3</sup>. In the residential areas tested, benzene ranged from 7.6 µg/m<sup>3</sup> (the area was near a metal working industry) to 19.3 µg/m<sup>3</sup> (the area had dense traffic). Suburban and rural levels were reported to be 7.0-7.7 µg/m<sup>3</sup> and, at three industrial sites, benzene ranged from 6.9-10.6 µg/m<sup>3</sup>. All these values were reported to be the annual arithmetic means from short sampling times (order of seconds). 24-Hour sampling periods in 1988-1989 showed the benzene concentrations in the Hamburg Elbtunnel to range from 80.5-95.3 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(157) (274) (827)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Urban atmospheric benzene concentrations were measured in The Netherlands in 1974 (2-hour sampling periods) and in 1982-1983 (annual averages from 80 2-hour samples collected/site). The average levels ranged from 1.6-41.8 µg/m<sup>3</sup> (with more than half of those measured being below 3 µg/m<sup>3</sup>) and the corresponding maxima ranged from 5.3-90.9 µg/m<sup>3</sup> (with half the values being below 11 µg/m<sup>3</sup>). The Maastunnel (traffic) was investigated in 1974 and the average benzene concentration was 79.8 µg/m<sup>3</sup>, with the maximum recorded level being 105.9 µg/m<sup>3</sup>. A further study in The Netherlands reported average and maximal concentrations from 1-hour samples taken in 1980 from rural (Island of Terschelling), urban (Delft) and heavily industrialised (near Rotterdam) sites. The average, over all three sites, ranged from 0.8-4.8 µg/m<sup>3</sup> and the maxima from 5.4-35.1 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(170) (455) (827) (1048)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In Stockholm, Sweden, average benzene atmospheric concentrations in the city centre were measured in 1982 and 1983 from eight 1-hour samples collected from each site over a 3-12 day period. In busy streets, the averages ranged from 44.3-147.7 µg/m<sup>3</sup> (minimum-maximum range 4.2-609.3 µg/m<sup>3</sup>); in light traffic the average was 14.6 µg/m<sup>3</sup> (range 4.6-48.2 µg/m<sup>3</sup>) and in a quiet street benzene averaged 7.7 µg/m<sup>3</sup> (range 3.2-20.9 µg/m<sup>3</sup>). At a recreation ground 12 km WNW of the city centre, benzene was detected at an average of 3.0 µg/m<sup>3</sup> (range 0.3-13.7 µg/m<sup>3</sup>). Two studies were conducted in 1981 of the atmospheres downwind of two car assembly plants. Benzene was detected at 5.1 and 3.1 µg/m<sup>3</sup> respectively.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(587) (827) (885)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In Zurich, Switzerland, benzene was detected, in 1971, at an average concentration of 172.3 µg/m<sup>3</sup> from samples taken at a distance of 300 m from the main road.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(444) (827)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** In a source dominated study, ambient air samples from 44 sites in 39 US urban areas were collected in electropolished, stainless steel canisters on week days from 6-9 am, between June and September 1984, 1985 and 1986. The samples were analysed by capillary gas chromatography with flame ionization detection to determine the C2-12 volatile hydrocarbon compositions. Benzene was detected in every sample at an overall average concentration of 40.2 µg/m<sup>3</sup>. When each site and year was considered individually the average benzene level ranged from 15.3-111.7 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004

(334) (530)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** In source dominated studies in the USA, concentrations of benzene in the atmosphere near chemical factories where benzene is used, ranged from 1.9-108.8 µg/m<sup>3</sup> and that at service stations ranged from 1 µg/m<sup>3</sup>-10.2 mg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004

(146) (352) (549) (834) (842) (1101)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** The rural background average concentration is reported to range from 0.32-54.33 µg/m<sup>3</sup>. Levels ranging from 0.03-1.85 µg/m<sup>3</sup> have been reported in rural areas of the Atlantic and Pacific Oceans, the Colorado Rockies, Norwegian Arctic, Pullman and Cape Meares. In five remote tropical sites, benzene ranged from undetectable to 5.74 µg/m<sup>3</sup>, with the range of averages between 0.22-2.07 µg/m<sup>3</sup>.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004

(442) (530) (549) (847) (1040)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** A survey of 80 surface water bodies (rivers and estuaries) was conducted across the UK between December 1988 and February 1989. The average level found in the freshwater samples which contained benzene at concentrations greater than the 0.1 µg/l detection limit, was 7.05 µg/l. Over half the samples collected (93 out of 154) contained less than the detection limit of benzene. A maximum level of 89.4 µg benzene/l was found in one sample.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(827) (989)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** Estuarine water samples taken from the River Tees in the UK, near to industrial effluent outfalls contained 0-5 µg benzene/l. The levels of benzene along the East coast of the UK were the subject of a survey in 1984. Samples were taken at a depth of 1 m from estuaries and benzene levels were reported to be 2-12 µg/l in the Humber Estuary, <1-18 µg/l in the Tees Estuary, 1-3 µg/l in the Tyne Estuary and <1-9 µg/l in the Thames Estuary.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(726) (827) (1250)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** In 1976, benzene levels were measured in the River Rhine. The average levels at Basle, Cologne and Duisburg were 0.2, about 0.2 and 0.8 µg/l respectively. Samples collected from the surface of Lake Zurich, Switzerland in October 1973 were reported to contain 0.028 µg benzene/l. In the same study, levels of 0.022 µg/l were detected in samples taken at a depth of 30 m and, in a sample of fresh spring water collected in the Zurich area in 1973, 0.018 µg benzene/l was detected.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(446) (768) (827)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** In 1975-1976, 14 heavily industrialized areas in the USA, with water basins, were investigated. It was found that 20% of the river samples contained between 1 and 7 µg/l. In the Brazos River, Texas, samples taken downriver of the outfalls from a chemical plant showed benzene levels of 0.004-0.910 µg/l.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(341) (530) (751) (827)

**Type of measurement:** background concentration

**Medium:** surface water

**Remark:** In 1975-1976, benzene levels in Lake Erie and Lake Michigan were reported to range from 0-1 µg/l (one of two sites being positive) and 0-7 µg/l (five of seven sites being positive) respectively. Levels in the Potomac River were found to be less than the 2 µg/l detection limit. In 1975, an average concentration of 5.4 µg/l was reported from samples taken from 700 random sites in the USA. The US EPA STORET database shows that benzene was detected in 15% of 1,271 samples measured and the median concentration was 5 µg/l.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004

(468) (530) (629) (641) (1085)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** A survey of the concentration of pollutants in urban stormwater runoff from 19 US cities was conducted. Benzene was not found frequently enough for its mean level in urban runoff to be calculated. Benzene was detected in 5% of the 86 samples taken and the range of detected concentrations was 3.5-13 µg/l.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(222) (827)

**Type of measurement:** background concentration

**Medium:** ground water

**Result:** Of the groundwaters of 32 public and private supply boreholes in the UK sampled in summer 1983, eleven were found to contain benzene. The mean level for these eleven was 0.027 µg/l, the average for all 32 samples was 0.009 µg/l and the maximum level was 0.07 µg/l. A further 59 industrial supply boreholes in the Birmingham aquifer which were not used for public supply, were assessed in 1986-1988. The entire sample area was urbanised and the boreholes were all contained within industrial premises. In general, the levels of non-chlorinated organic compounds were low; the maximum benzene level found being 0.6 µg/l.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(612) (827) (946)

**Type of measurement:** background concentration

**Medium:** ground water

**Result:** Benzene levels in European groundwater have been reported as 0.045 µg/l in Zurich, Switzerland and 0.005-0.030 µg/l in unpolluted areas in The Netherlands.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(445) (827) (1048)

**Type of measurement:** background concentration

**Medium:** sediment

**Remark:** Surface sediment taken from two sites in Walvis Bay, near Capetown, South Africa, contained 0.2-20.4 µg/kg (dry weight). Estuarine sediments in the Tees Estuary, UK, were found to contain benzene at levels of 1.3-3.9 µg/kg. In May and June 1980, sediment was collected from Lake Pontchartrain, Louisiana, USA. Benzene levels of 8-21 µg/kg (wet weight) were found. The US EPA STORET database reports 9% of 355 samples to contain benzene at a median concentration of <5 ppb.

Used for Risk Assessment.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

16-MAR-2004

(355) (530) (827) (1085) (1248) (1250)

**Type of measurement:** background concentration

**Medium:** food

**Remark:** Benzene has been detected in fruits, nuts, vegetables, dairy products, meat, fish, poultry, eggs and beverages. It has been reported to be present in the aroma from cooked fresh ground beef and fresh and frozen cod fillets. Actual levels of 2 µg/kg in heat treated/canned beef, 120 µg/kg in Jamaican rum, 500-2100 µg/kg in eggs and 19 µg/kg in irradiated beef have been reported. Benzene was detected in all eight samples of mother's milk from four US urban areas.

Used for Risk Assessment.

**Source:** BP Chemicals Ltd LONDON;

German Rapporteur

16-MAR-2004

(530) (549) (725) (817) (827) (881) (1192) (1266)

**Type of measurement:** background concentration

**Medium:** drinking water

**Remark:** There is one report of a benzene concentration of 0.1 µg/l in Czechoslovakian drinking water and, in the USA, levels of 0.1-0.3 µg/l have been reported. In five US cities, tested in 1974-1975, levels were found to range from 0-0.3 µg/l and of 113 public supplies in 1976, seven contained benzene, the average concentration being <0.2 µg/l. Three surveys of community water were conducted. In the first, none of the 111 samples contained benzene; seven of the 113 in the

second were positive (mean 4 µg/l) and four of the 16 in the third were positive (0.95 µg/l maximum). The US Groundwater Supply Survey in 1982 looked at 466 samples of finished drinking water taken randomly from the 1000 samples in the survey. Benzene was detected in 0.6%; the median level being 3 µg/l and maximum being 15 µg/l. Of 1174 community and 617 private drinking water wells in Wisconsin tested in June 1984, benzene was detected in 0.34 and 2.9% respectively.

**Source:** BP Chemicals Ltd LONDON;  
German Rapporteur  
16-MAR-2004 (141) (223) (248) (530) (549) (643) (842) (1191)

**Type of measurement:** background concentration

**Medium:** biota

**Result:** Measurements were made of oysters and clams collected in May and June 1980 from Lake Pontchartrain, Louisiana. In one sample of oysters (*Crassostrea virginica*) benzene was found at a level of 220 µg/kg (wet weight) and in clams (*Rangia cuneata*) at 260 µg/kg (wet weight). In a second sample of clams collected from the same area, benzene could not be detected however, the detection limits were not reported.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment  
16-MAR-2004 (355) (827)

**Type of measurement:** background concentration

**Medium:** biota

**Remark:** Two species of macroalgae, *Ulva lactuca* and *Hypnea musciformis* (seaweeds) were grown in tanks using circulating seawater. Benzene was detected in the algae at 20 µg/kg (dry weight).

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004 (1249)

**Type of measurement:** background concentration

**Medium:** biota

**Remark:** Marine fish and invertebrate samples were collected 6 km from the outfall from the Los Angeles County wastewater treatment plant. Benzene was present at levels ranging from <1 µg/kg (wet weight) in the liver of Pacific sanddab and in the muscle of ridgeback prawn, up to 52 µg/kg (wet weight) in the liver of Dover sole.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

16-MAR-2004 (432)

**Type of measurement:** background concentration

**Medium:** air

**Result:** From January to March 1994, airborne benzene levels (24-h averages) were measured at three different sites in the city of Munich/Germany giving following results: 0.72-1.55 ug/m<sup>3</sup> (average: 0.96 ug/m<sup>3</sup>; outskirts with very low traffic density, some 100 m away from roads), 15.63-24.89 ug/m<sup>3</sup> (average: 20.44 ug/m<sup>3</sup>; street with heavy traffic, about 26000 vehicles/d), 2-9 ug/m<sup>3</sup> (average: 5 ug/m<sup>3</sup>; suburban residential area) (TUEV Umwelttechnik, 1994; DEKRA Umwelt, 1994).  
In 1992 and 1993, the benzene concentrations in seven German cities ranged between 6 and 45 ug/m<sup>3</sup> (monthly averages) (LUEN/UBA, 1993). In the state of Baden-Wuerttemberg monthly average values of 15-28 ug/m<sup>3</sup> with an annual average of 20 ug/m<sup>3</sup> were found in 1992/93 (UMEG, 1994).  
In April 1986-1987, 12 different sites in and around Hamburg, West Germany, were assessed for their atmospheric benzene concentrations. In a downtown residential area near a street with dense traffic a maximum value of 19.3 ug/m<sup>3</sup> was found. The minimum value was 7.0 ug/m<sup>3</sup> in a suburban area in rural surroundings. In two purely industrial regions the benzene levels were 6.9 and 10.6 ug/m<sup>3</sup> respectively. In three residential areas industrial regions in the neighbourhood, benzene concentrations between 7.6 and 17.7 ug/m<sup>3</sup> were determined. All these values were reported to be the annual arithmetic means from short sampling times (order of seconds) (Bruckmann et al., 1988).  
24-hour sampling periods in 1988-1989 showed the benzene concentrations in the Hamburg Elbtunnel to range from 80.5-95.3 ug/m<sup>3</sup> (Dannecker et al., 1990).

**Source:** Deutsche Shell Chemie GmbH Eschborn;  
German Rapporteur

16-MAR-2004 (157) (274) (291) (705) (1166) (1186)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** Compilation of literature data

**Result:** The Netherlands:  
The average levels of airborne benzenes in cities in the early 80s ranged from 1.6-9.0 ug/m<sup>3</sup> and the corresponding maxima which mainly occurred in winter ranged from 5.3-59.4 ug/m<sup>3</sup>. The annual large scale concentration level is estimated to be ca. 2 ug/m<sup>3</sup>. For cities, the concentrations are by a factor of 2 to 10 higher.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004 (1048)

**Type of measurement:** background concentration

**Medium:** surface water

**Remark:** Compilation of literature data.

**Result:** United Kingdom:

(a) Surface water:

In 1988/89, a survey of 80 surface water bodies (rivers and estuaries) gave an average benzene level found in the freshwater samples which contained benzene at concentrations greater than the 0.1 ug/l detection limit of 7.05 ug/l. About 60% of the samples collected contained less than the detection limit of benzene. A maximum level of 89.4 ug benzene/l was found in one sample.

(b) Sea water:

The benzene levels measured in a depth of 1 m along the Eastcoast were 1984 in the range of < 1 - 18 ug/l (Humber, Tees, Tyne and Thames estuaries)

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(827)

**Type of measurement:** background concentration

**Medium:** ground water

**Remark:** Compilation of literature data.

Used for Risk Assessment.

**Result:** The Netherlands:

In the early 80s benzene concentrations of 0.005-0.030 ug/l were found in unpolluted areas.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(1048)

**Type of measurement:** background concentration

**Medium:** biota

**Result:** Four species of macroalgae, *Ulva lactuca*, *Ascophyllum nodosum*, *Gracilaria tikvahiae* and *Hypnea musciformis* (seaweeds) were grown in tanks using circulating seawater. Benzene was detected in two species (*Ulva lactuca*, *Hypnea musciformis*) at 20 ug/kg (dry weight). The authors suggest that the seaweeds are a possible source of the volatile organic compounds identified in recent marine sediments.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(1249)

**Type of measurement:** background concentration**Medium:** ground water**Remark:** Compilation of literature data.**Result:** United Kingdom:

In the 80s an average benzene concentration of 0.009 ug/l was found in a survey of public and private supply boreholes. The maximum level was 0.07 ug/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(827)

**Type of measurement:** background concentration**Medium:** air

**Result:** From 1986-1990, three month running mean concentrations of benzene at a rural site in the UK (Harwell, Oxfordshire) were between about 0.4 ppb (1.3 ug/m<sup>3</sup>) and 1.7 (5.5 ug/m<sup>3</sup>). The minimum values were always detected during the months June - September, the maximal concentrations were found during November - February.

From 1991-1992, monthly mean concentrations of benzene in Central London (Exhibition Road) were between about 1 ppb (3.25 ug/m<sup>3</sup>) and 14 ppb (45.5 ug/m<sup>3</sup>; smog episode in winter) with an annual average of about 4 ppb (13 ug/m<sup>3</sup>).

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(1179)

**Type of measurement:** background concentration**Medium:** other: indoor air**Remark:** Reference (Krause et al., 1987) not available**Result:** A comparison of indoor/outdoor benzene levels in 52 private homes in the city of Munich, FRG gave following results:

concentration (ug/m <sup>3</sup> )	min	max	mean
indoor	0.41	17.40	2.74
outdoor	0.27	22.26	2.68

(detection limit: 0.005 ug/m<sup>3</sup>; sampling period not given)  
From comparison with indoor and outdoor NO<sub>x</sub> concentrations the authors conclude that the main source is the infiltration of polluted outdoor air (Gebefuegi et al., 1994).

A survey of 479 German households in 1985/86 gave following indoor air benzene levels:

range: < 1-90 ug/m<sup>3</sup>non-smokers: 6.5 ug/m<sup>3</sup>smokers: 11 ug/m<sup>3</sup> (Krause et al., 1987)**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

16-MAR-2004

(396) (640)

**Type of measurement:** background concentration

**Medium:** surface water

**Result:** From 1990-1992, benzene was not detected in the river Rhine (1990: sampling sites not given; 1991/92: sampling site: Lobith, The Netherlands) (detection limit 0.1 ug/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(554) (948) (949)

**Type of measurement:** background concentration

**Medium:** air

**Result:** From 1992-1993, following annual average benzene levels were found at different sampling sites in Austria:

two rural areas 1.0/1.8 ug/m3

Vienna/low traffic density 2.0/3.2 ug/m3

Vienna/high traffic density 8.8-15.5 ug/m3

16.0 ug/m3 (service station)

Lobau/petrol depot 12.7-17.0 ug/m3 (dependent on distance)

(detection limit 0.8 ug/m3)

Measurements of airborne benzene levels in Vienna/Austria and its surroundings from October 1986 - February 1987 and in May 1987 respectively gave following results (arithmetic means each):

locations with heavy traffic: 15.6-179 ppbC (5 sampling sites)

residential areas: 13.1 ppbC (2 sampling sites)

wood (about 10 km from Vienna): 6.9 ppbC (1 sampling site)

(detection limit: 0.1-0.2 ppbC from a 1l air sample)

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(478) (663)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In Italy the benzene level in air of three large cities (Rome, Milan, Taranto), one suburban site (Montelibretti near Rome) and one forest site (Monti Cimini, pine forest) was determined during day-time (9-11 a.m.) in mild seasons (sampling period not given). The sampling lasted at least 15 d. Following average values were found: 39.0-43.9 ug/m3 (cities), 1.84 ug/m3 (suburban site), 4.39 ug/m3 (forest).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(205)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Airborne benzene concentrations were detected 1991 and 1992 in two wooded areas of Europe (near Storkow/former GDR southeast of Berlin in a pine forest and 25 km west of Rome/Italy in an area with mediterranean macchia) and at a height of 5050 m at the foot of the Mount Everest/Nepal. The concentrations in Europe were 0.54 ug/m<sup>3</sup> (former GDR) and 3.08 ug/m<sup>3</sup> (Italy) respectively (one measurement each). In Nepal, levels between 0.27 and 1.99 ug/m<sup>3</sup> were found. The authors concluded from their results that the airborne benzene in the Himalaya region is due to long-range transport from polluted areas.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(206)

**Type of measurement:** background concentration

**Medium:** other: comparison indoor/outdoor air

**Result:** From 1979-1988, the personal exposures for benzene in 5 US cities were between 4 and 18 ug/m<sup>3</sup> (median 24-h averages). The arithmetic mean values were larger, ranging from 6-30 ug/m<sup>3</sup> with a grand mean of about 15 ug/m<sup>3</sup>. These personal exposure exceeded the outdoor concentrations (range of median values: 2-16 ug/m<sup>3</sup>; arithmetic means: 2-19 ug/m<sup>3</sup> with a grand mean of ca. 6 ug/m<sup>3</sup>). The measured maximum concentrations differ even more. For indoor 24-h personal exposures 30-250 ug/m<sup>3</sup> were measured compared with 6-35 ug/m<sup>3</sup> for outdoor maxima. Breath measurements identified smoking as the most important single source of benzene exposure. Smokers had mean breath concentrations of 15 ug/m<sup>3</sup> whereas in the breath of non-smokers benzene concentrations of 1.5-2 ug/m<sup>3</sup> were found.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(1225)

**Type of measurement:** background concentration

**Medium:** food

**Result:** A survey of 50 US American foods and beverages (sampling period 1991-1992) gave following results:  
(a) foods with no added benzoates:  
benzene not detected: strained apple juice, cranberry juice concentrate, raspberry drink, cola soda, brewed instant coffee, strawberry preserves, grape jelly, liquid beef bouillon, raw and baked potato, ground nutmeg, fried egg, fresh tomato  
benzene level < 1 ug/kg: apple juice, strained apple-cherry juice, cranberry juice cocktail (2 different brands), fresh cranberries, grape drink, fruit punch, orange soda, hard-boiled egg, smoked fish (chub), roasted peanuts  
benzene level >= 1 ug/kg: cranberry juice cocktail (2 different brands; 1 ug/kg), red raspberry preserves (1 ug/kg), black currant preserves (2 ug/kg), liquid smoke (2

different brands; 21 and 121 ug/kg)  
(b) foods with added benzoates:  
benzene not detected: diet orange soda (1 brand), soy sauce  
(2 different brands), iced tea  
benzene level < 1 ug/kg: fruit punch, diet cola, diet orange  
(1 brand), diet white grape soda, pickled vegetables, citrus  
salad, diet raspberry soda, lite strawberry preserves, sweet  
relish, salad peppers, lite syrup product  
benzene level >= 1 ug/kg: Bloody Mary mix (3 ug/kg),  
imitation grape jelly (5 ug/kg), different sauces (1-22  
ug/kg), Margarita mix (2 ug/kg), diet cherry berry soda (2  
ug/kg), diet grapefruit soda (1 ug/kg), imitation strawberry  
preserves (38 ug/kg), lite orange marmalade (1 ug/kg),  
litegrape jelly (1 ug/kg),

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(759)

**Type of measurement:** background concentration

**Medium:** air

**Result:** In summer 1991 and winter 1992 benzene levels in a Swedish  
autotunnel (Tingstad tunnel, Goeteborg) were determined. The  
summer values were 61 and 450 ug/m<sup>3</sup> the second one being  
caused by slow traffic in both directions of the tunnel  
tube. In winter, 104-301 ug benzene/m<sup>3</sup> were found. The  
proportion of benzene was about 7% of the total non-methane  
volatile hydrocarbons.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(71)

**Type of measurement:** background concentration

**Medium:** food

**Result:** Benzene was detected in US American chicken meat, ham and  
pork samples by irradiation in concentrations between 5 and  
12 ug/kg.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(1041)

**Type of measurement:** background concentration

**Medium:** other: indoor air

**Result:** Benzene was found in the air of new and recently renovated  
houses in Switzerland in a maximum concentration of 20  
µg/m<sup>3</sup>. 10% of the measured levels were < 5 µg/m<sup>3</sup>, 50% were  
max. 7 µg/m<sup>3</sup>, 90% were max. 14 µg/m<sup>3</sup>, and the maximum found  
was 20 µg/m<sup>3</sup>.

As the substance was detected in all of the examined houses  
and the ratio to outdoor air is almost 1 the authors  
concluded that it is mainly infiltrated from outdoor air.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(969)

**Type of measurement:** background concentration**Medium:** other: human blood**Result:** In 1991, the benzene content of the blood of 55 year old women who were not occupationally exposed to benzene and 6 year old children living either in a large city or in a rural area was determined in the state of Nordrhein-Westfalen/FRG. The geometric mean values were as follows:

	large city	rural area
women	0.072 ug/l	0.048 ug/l
children	0.143/0.133 ug/l*	0.128 ug/l

\* 2 different cities

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(777)

**Type of measurement:** background concentration**Medium:** surface water**Remark:** Compilation of literature data**Result:** The Netherlands:

(a) surface water: in the 80s, the benzene concentrations of surface waters (rivers and lakes) generally were &lt; 0.1 ug/l. In the Rhine a median concentration of 0.026 ug/l were found.

(b) sea water: in the 80s, &lt; 0.005-0.02 ug/l benzene were detected in the coastal parts of the North Sea. In the central part of the North Sea average concentrations of about 0.005 ug/l were found.

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(1048)

**Type of measurement:** background concentration**Medium:** air**Remark:** Compilation of literature data.**Result:** United Kingdom:

In the 80s, the airborne benzene levels measured mainly in or near London ranged between 0.2 and 181.8 ug/m3 with average values between 5.2 and 28.1 ug/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(827)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** Compilation of literature data

**Result:** USA:  
In the 80s, airborne benzene levels ranging from 0.3-210 ug/m3 were found in the large cities (average values: 4.6-19ug/m3). In a rural area the concentrations were 0.07-2.8 ug/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(56)

**Type of measurement:** background concentration

**Medium:** air

**Remark:** Compilation of literature data.

**Result:** Former FRG:  
In the 80s, the airborne benzene levels in cities ranged between 0.4 and 171.8 ug/m3 with average values of 7.5-51.3 ug/m3. In rural areas concentrations of about 1 ug/m3 were found.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(162)

**Type of measurement:** background concentration

**Medium:** surface water

**Remark:** Compilation of literature data.

**Result:** Former FRG:  
In the 80s, benzene concentrations between 0.01 and 0.40 ug/l were found in rivers and lakes.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(162)

**Type of measurement:** background concentration

**Medium:** air

**Result:** The benzene concentration measured inside an automobile during urban and suburban travel ranged from 38 to 46 ug/m3.

The benzene concentration measured inside a stationary automobile with a diesel engine ranged from 3.2 to 3.5 ug/m3.

The benzene concentration measured inside a stationary automobile with a gasoline engine ranged from 12 to 33 ug/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(301)

**Type of measurement:** background concentration**Medium:** air**Result:** Benzene concentrations in air at the perimeter of petroleum refineries ranged from < 0.001 to 0.023 mg/m<sup>3</sup> based upon measurements at 3 refineries for 24-hour periods.**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(28)

**Type of measurement:** background concentration**Medium:** air**Result:** Benzene measured in the air of German homes with smokers ranged from 10 to 12 ug/m<sup>3</sup>. German homes with no smokers were measured at 6.5 ug/m<sup>3</sup>.**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(330)

**Type of measurement:** background concentration**Medium:** air**Result:** European chemical producers (N = 14) provided worker air exposure data for a range of work categories. The data presented are the results of over 1000 measurements.

(as mg/m <sup>3</sup> )	Minimum	Maximum	Ave
Benzene Production	0.002	16	0.87
Benzene Use	0.03	5.4	0.63
Transportation	0.01	266	0.86
Maintenance	0.06	62	0.62
Laboratory	0.03	7.1	0.79
Handling	0.1	40	1.5
Storage	0.1	5.9	0.91
Waste Operations	0.003	3.1	0.34
Operations	0.06	43	5.8

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur**Flag:** Risk Assessment

16-MAR-2004

(181)

**Type of measurement:** background concentration**Medium:** air**Result:** Air measurements of automobile mechanic exposures at servicestations with unvented exhaust gasses ranged from 0.03 to 2.8 mg/m<sup>3</sup> for short term exposures (mean = 0.48) and from 0.03 to 1.5 mg/m<sup>3</sup> for full shift samples (mean = 0.51).**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(236)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Personnel exposures to benzene in air for coke oven operations: coke battery personnel 0.99 mg/m<sup>3</sup> (0.31 ppm) and by-product plant personnel 4.2 mg/m<sup>3</sup> (1.3 ppm).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(312)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Benzene in air measurement from nitrobenzene manufacture ranged from 0.003 to 0.011 mg/m<sup>3</sup>.

Benzene in air measurement from cumene manufacture ranged from 0.025 to 0.051 mg/m<sup>3</sup>.

Benzene in air measurement from maleic anhydride manufacturer ranged from 0.002 to 0.032 mg/m<sup>3</sup>.

Benzene in air measurement from nitrobenzene manufacture ranged from 0.006 to 0.035 mg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(352)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Benzene in air measurements were 3 and 8 ug/m<sup>3</sup> respectively for petroleum and diesel powered taxi cabs under highway conditions.

Benzene in air measurements ranged from 40 to 110 ug/m<sup>3</sup> in diesel taxi cabs during city rush hour traffic.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(521)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Benzene in air measurements ranged from < 0.9 to 12 mg/m<sup>3</sup> in various short term tasks with direct petroleum contact.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(654)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Average indoor air concentration of benzene reported as 10 ug/m3 for 319 homes in the Netherlands.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(675)

**Type of measurement:** background concentration

**Medium:** air

**Result:** The measured exposures of Swedish workers in the workplace indicate that workers may temporarily be exposed to air concentrations above the 8h TWA.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(840)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Show making operations have a history of significant benzene exposures. A 1964 survey showed measurements ranging

from 130 to 140 mg/m3 (41 to 44 ppm).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(812)

**Type of measurement:** background concentration

**Medium:** air

**Result:** A co-operative project among coal coke producing companies produced data on the concentration of benzene across a range of coke oven operations for several different companies. The concentrations range from 0.04 to 31 mg/m3. Outside the perimeter of the plants the concentration ranged from 0.006 to 0.063 mg/m3 and varied based upon distance.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(1126)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Breathing zone benzene exposures ranged from < 0.3 to 18.9 mg/m3 (<0.09 to 5.9 ppm) in the US tire industry.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(1203)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Mean exposures for refinery operations were: operators 0.58mg/m<sup>3</sup> (0.18 ppm), off-site workers 1.8 mg/m<sup>3</sup> (0.56 ppm), and maintenance staff 2.5 mg/m<sup>3</sup> (0.78 ppm). Exposures to others in distribution, etc are provided.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(240)

**Type of measurement:** background concentration

**Medium:** air

**Result:** For petroleum drumming operations, the mean exposures was 27mg/m<sup>3</sup>. Self-service fueling ranged from ND to 13.13 mg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(237)

**Type of measurement:** background concentration

**Medium:** air

**Result:** The estimated benzene absorbed daily dose for occupationally and non-occupationally exposed population ranged from 74 to 8424 ug/day.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(235)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Ambient benzene concentrations in air ranged from 0.0016 to 0.119 mg/m<sup>3</sup> near service stations.

Ambient benzene concentrations in air ranged from 0.0011 to 0.0933 mg/m<sup>3</sup> near petroleum distribution terminals.

Ambient benzene concentrations in air ranged from < 0.001 to 0.023 mg/m<sup>3</sup> near refineries.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(241)

**Type of measurement:** background concentration

**Medium:** air

**Result:** Smoking adds about 5-12 ug/m<sup>3</sup> of benzene to the indoor environment. The information was gathered through monitoring and surveys of 113 people in Germany.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(515)

**Type of measurement:** background concentration

**Medium:** drinking water

**Result:** Benzene measurements of tap water in Spain were < 0.05 ug/L.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(453)

**Type of measurement:** background concentration

**Medium:** drinking water

**Result:** Benzene measurements of drinking water in Canada ranged from < 0.1 to 0.2 ug/L.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(861)

**Type of measurement:** background concentration

**Medium:** food

**Result:** The concentration of benzene measured in various foods:

Fish	<6e-05, ug/g MDL
Stem of Plants	<3e-05, ug/g MDL
Root of Plants	<9e-05, ug/g MDL
Meat	<7e-05, ug/g MDL
Milk	<2e-05, ug/g MDL
Other	0.03 ug/g

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(29)

**Type of measurement:** background concentration

**Medium:** air

**Method:** Air samples (3 - 6 l) were sampled in silica-coated stainless steel canisters 4 times a day and analysed by capillary-GC-FID after a 3-stage preconcentration process using external standards for identification and quantification.

**Result:** Results in pptv / ug/m<sup>3</sup>:  
Range of values: 75 - 176 / 243 - 570;  
average ± standard deviation: 119 ± 24 / 386 ± 78.  
The minimum observed in this period was reached on 18th December, the maximum on 19th December.

**Source:** German Rapporteur

**Test condition:** Air sampling took place from 12th to 20th December 1999

during the winter in which Chichi-jima Island is down-wind from East Asia and thus influenced by long-range transport of air pollutants from the East Asian mainland.

16-MAR-2004

(605)

**Type of measurement:** background concentration**Medium:** air

**Method:** Air was sampled by pressuring it into stainless steel canisters. In the Laboratory, the air was dried, kryofocussed twice (on a thermal desorber and on the GC column) and analysed by capillary-GC-FID or capillary-GC-MS.

**Remark:** This work was primarily concerned with the emissions of biogenic compounds (e. g. isoprene) from vegetation in a rural area of Greece.

**Result:** Benzene concentrations found during the 5 sampling periods (ppb /  $\mu\text{g}/\text{m}^3$ ):

Sampling period	mean $\pm$ standard deviation	median	minimum maximum
(1)+(5)	$0.03 \pm 0.02/0.10 \pm 0.06$	0.02/0.06	0.01/0.03
(2)+(3)	$0.08 \pm 0.06/0.26 \pm 0.19$	0.07/0.23	0.02/0.06
(4)	$0.17 \pm 0.10/0.55 \pm 0.32$	0.12/0.39	0.09/0.29

**Source:** German Rapporteur

**Test condition:** Air samples were collected on the north-west of the Peloponnese peninsula at the slope of a mountain at a height of 1070 m from April 1996 to May 1997. In the measuring periods, 1 hour samples were taken every day at 13-14 hours local time.

The sampling periods were:

- (1) 29 April - 4 May 1996,
- (2) 25 - 30 June 1996,
- (3) 2 - 10 September 1996,
- (4) 28 October - 4 November 1996, and
- (5) 5 - 22 May 1997.

16-MAR-2004

(796)

**Type of measurement:** background concentration**Medium:** air

**Result:** A large number of cities in the USA has been studied for their atmospheric benzene concentrations. In general, the averages ranged from 0.4  $\mu\text{g}/\text{m}^3$  in an industrial area of North Carolina where there is no chemical industry (1982 measurements; 12-hour outdoor samples collected) to 47.85  $\mu\text{g}/\text{m}^3$  in Los Angeles in 1966. The majority of these readings were below 20  $\mu\text{g}/\text{m}^3$ . The lowest level was 0.2  $\mu\text{g}/\text{m}^3$  measured between June and July 1979 in Oakland, California by 24-hour, 7 days/week continuous sampling schedules. The highest level was reported to be 431.33  $\mu\text{g}/\text{m}^3$  measured in Rutherford, an industrial area in New Jersey, from six 20-minute samples collected on 3 consecutive days and repeated over 4-9 weeks.

**Source:** BP Chemicals Ltd LONDON

**Flag:** German Rapporteur  
Risk Assessment  
07-APR-2004 (136) (137) (335) (530) (827) (896) (1039) (1221)

**Type of measurement:** background concentration

**Medium:** ground water

**Result:** A survey was conducted of the groundwater from 466 randomly selected and 479 US state agency selected sampling sites. Of those sites serving fewer than 10,000 people, 0.4 and 1.6% contained benzene respectively; the median levels for the positive sites being 0.61 and 1.6 µg/l respectively and the maximum levels being 0.61 and 12 µg/l respectively. Of those sites serving >10,000 people, 1.1 and 1.9% contained benzene respectively; the median levels for the positive sites being 9.0 and 2.7 µg/l respectively and the maximum levels being 15 and 12 µg/l respectively. Groundwater sampled from eight monitoring wells in South Ontario, Canada where bituminous and petroliferous sedimentary rocks are expected to influence the aquifer resource, was found to contain levels of up to 100 µg benzene/l. Benzene was reported in 3.2% of 63 private supply wells in Nebraska in 1982 at a median level of 1.6 µg/l and a maximum of 1.8 µg/l. Levels of 30-330 µg/l were reported in groundwater samples from Connecticut, New Jersey and New York and a maximum of 0.1 µg/l was found in sewage-contaminated groundwater from a site at Otis Air Base, Boston, Massachusetts.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

07-APR-2004 (70) (430) (827) (921) (1045) (1247)

**Type of measurement:** concentration at contaminated site

**Medium:** ground water

**Result:** Samples from a chalk aquifer near to a petrol storage facility in East Anglia, UK were analysed for benzene. At distances of 210, 120 and 10 m from the storage facility, benzene levels were found to be 1-10 µg/l, >250 µg/l and 1250 µg/l respectively.

**Source:** BP Chemicals Ltd LONDON

German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004 (530) (827) (1121)

**Type of measurement:** concentration at contaminated site

**Medium:** ground water

**Result:** The benzene levels in groundwater sampled at a site on a solvent recovery plant in Wisconsin were reported to be 12,000 µg/l at the water-table and <10 µg/l (below the detection limit) at depth. At a sampling site 80 m downgradient from the recovery plant, levels of <10 µg/l (below the detection limit) and 20 µg/l were recorded respectively.

**Source:** BP Chemicals Ltd LONDON

**Flag:** German Rapporteur  
Risk Assessment  
16-MAR-2004 (217) (827)

**Type of measurement:** concentration at contaminated site  
**Medium:** soil

**Remark:** A study of soil near US factories where benzene was either used or produced found levels of benzene in the soil of <2-191 µg/kg.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur  
29-JUL-2002 (352) (549)

**Type of measurement:** concentration at contaminated site  
**Medium:** other: effluents from service stations and petrol depots

**Result:** Monthly sampling from November 1983 to May 1984 in eight service stations and two petrol depots in the former FRG gave following average benzene levels:  
4 service stations with car-wash: 1.4-5.5 ug/l  
1 service station with garage: 70.65 ug/l  
2 petrol depots: 0.05/1267 ug/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
29-JUL-2002 (85)

**Type of measurement:** concentration at contaminated site  
**Medium:** other: leachate from sanitary landfill site

**Result:** Benzene in leachate from the sanitary landfill site in Hamburg-Georgswerder/former FRG was detected 1981-1982 at 4 sampling sites in a concentration range from not detectable (limit of detection not given) to 96 ug/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
29-JUL-2002 (418)

**Type of measurement:** concentration at contaminated site  
**Medium:** air

**Result:** Measurements of VOC concentrations in the emissions of three different coal-fired power stations in France showed benzene levels between 3.1 and 20 ug/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment  
16-MAR-2004 (393)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** In moped exhaust benzene had a proportion of 5.1% (w/w) related to the non-methane hydrocarbons (for comparison: benzene content of the fuel 4.2% (w/w)) during a test distance of 700-800 m on plain ground at the maximum speed of 30 km/h if conventional unleaded reformat-based grade fuel was used. With alkylate-based petrol the benzene content of the exhaust was reduced to 0.10% (w/w) (benzene content of the fuel 0.013% (w/w)).  
In exhaust from a lawn mower operated with conventional unleaded reformat-based grade fuel the benzene content was 7% (w/w). Using alkylate-based petrol the benzene level was reduced to 0.5% (w/w) (= 6 ug/m3).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(852)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** In 1987, benzene was found in stack gases of four US American municipal wastewater sludge incinerators in amounts between 0.107 g/h (plant with afterburning device) and 7.87g/h under steady state conditions.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(871)

**Type of measurement:** concentration at contaminated site

**Medium:** other: influent and effluent of a municipal wastewater treatment plant

**Result:** From 1989-1991 benzene levels in the influent and the effluent of the municipal wastewater treatment plant of Goeteborg/Sweden were determined. The influent range was found to be 0.1-5 ug/l with average concentrations of 3.9 ug/l (1989), 3.5 ug/l (1990) and 0.3 ug/l (1991). The effluent concentrations were generally < 0.5 ug/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(875)

**Type of measurement:** concentration at contaminated site

**Medium:** soil

**Result:** Benzene was found in the soil of 2 gas works stations in the Netherlands in concentrations of 1 and 2 mg/kg respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(377)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** In winter 1985 and summer 1986, airborne benzene concentrations at 2 petrol stations in Munich/Germany were between 462 and 27170 ug/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(953)

**Type of measurement:** concentration at contaminated site

**Medium:** other: inner rooms of automobiles

**Result:** In the inner rooms of automobiles average benzene concentrations of 40-60 ug/m<sup>3</sup> were derived from literature values (Eikmann, 1991). Under unfavourable conditions (parking in the sun, closed windows) maximum levels up to 2700 ug/m<sup>3</sup> were found (Muecke et al., 1984).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

16-MAR-2004

(325) (799)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** Simultaneous sampling in stainless steel canisters (whole air samples for analysing C<sub>2</sub> - C<sub>12</sub> hydrocarbons, 6 l capacity) and on Tenax-TA adsorbent (for analysing C<sub>8</sub> - C<sub>20</sub> hydrocarbons; sampling rate: 0.5 - 0,7 l/min). The hydrocarbons from the whole air samples were analysed by cryofocussing and capillary GC-FID (quantification) or GC-infrared detection (IRD) / MS (identification).

The hydrocarbons from the Tenax adsorbents were thermally desorbed and analysed by cryofocussing and capillary GC-FID.  
**Remark:** Comprehensive description with many experimental details including the stability of the samples in terms of storage time.

**Result:** Fort McHenry Tunnel:  
7.3 - 188.5 ppbC = 1.2 - 31.4 ppbV = 3.9 - 102 µg/m<sup>3</sup>  
Tuscarora Tunnel:  
4.6 - 35.0 ppbC = 0.8 - 5.8 ppbV = 0.3 - 18.9 µg/m<sup>3</sup>

**Source:** German Rapporteur

**Test condition:** Measurements in two tunnels:  
1. Fort McHenry Tunnel, Baltimore, Maryland: Sampling in the ventilation ducts of the tunnels for 11 h per run during the period 18 - 24 June 1992 gave 10 samples each.  
2. Tuscarora Tunnel, Pennsylvania turnpike: Sampling at the two ends of the tunnel with ventilation turned off for 11 h per run during the period 2 - 8 September 1992 gave 4 samples each.

**Test substance:** No data

16-MAR-2004

(1313)

**Type of measurement:** concentration at contaminated site

**Medium:** other: landfill gas

**Method:** Sampling by drawing 25 ml of gas through Tenax GC (for non-polar species) or Porapak Q (for acid and neutral compounds and low molecular-weight compounds) or 7.5 l gas through a cold trap (- 80 °C) (for water-soluble compounds). Samples on Tenax GC were thermally desorbed, cryofocussed, and analysed by capillary GC-MS. Samples on Porapak Q were cooled to - 80 °C and then desorbed with helium directly to a packed GC column (2 mm x 1 m, Chromosorb 101) with MS detection. Samples of condensate were also analysed by GC-MS (packed column, 2 mm x 1 m, Chromosorb 101).

**Remark:** Paper on the development of a method of analysis of landfill gases with a few examples of real sample analyses.

**Result:** Of the six sites analysed, data of one site are presented. Benzene was found at 5 mg/m<sup>3</sup>.

**Source:** German Rapporteur

**Test condition:** Gas samples were collected on-site using bore-holes about 0.9 - 4.3 m deep into the landfills. Details on the location of the landfills or the time of sampling were not given.

**Test substance:** No data

16-MAR-2004

(153)

**Type of measurement:** concentration at contaminated site

**Medium:** surface water

**Result:** The monitoring data for the river Elbe show that between the sampling stations Boizenburg (river km 559.0), Zollenspieker (river km 598.7), where the city of Hamburg is situated, and Grauerort (river km 660.5; situated in the North Sea estuary) the benzene concentration increased from 1 to 5 and then decreased to 0.5 µg/l.

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(40)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** Annual means in 1995 and 1996: 5 µg/m<sup>3</sup>.  
Location: Frankfurt/Main street.  
(No further details available on type and extend monitoring or monitoring site.)

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(507)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Remark:** Annual averages, lowest and highest monthly averages and maximum 30 minute-averages in 2000 ( $\mu\text{g}/\text{m}^3$ ):

Frankfurt/Main, Friedberger Landstr.: 4 / 2 / 5 / 27  
Wiesbaden, Ringkirche: 5 / 4 / 7 / 34  
Kassel, Fünffensterstr.: 6 / 5 / 7 / 49

Passive monitoring programme on benzene 2000:  
Annual means for individual streets in different cities in Southern Hestia ( $\mu\text{g}/\text{m}^3$ ):

Frankfurt (16 streets): 2.3 - 3.9  
Offenbach (3 streets): 2.7 - 3.3  
Neu-Isenburg (1 street): 3.9  
Wiesbaden (13 streets): 2.5 - 5.0  
Darmstadt (5 streets): 2.5 - 4.0  
Dreieich (2 streets): 2.7 - 3.0

**Source:** German Rapporteur

29-JUL-2002

(509)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** Benzene concentrations (average / minimum / maximum) ( $\mu\text{g}/\text{m}^3$ ):

Duesseldorf, Cornelius st.: 13.5 / 1.7 / 35.5  
Essen, Hindenburg st.: 11.6 / 3.3 / 29.7  
Duesseldorf-Moersbroich: 15.5 / 6.3 / 29.4  
Essen East: 8.9 / 3.2 / 23.6

**Source:** German Rapporteur

**Test condition:** Monitoring of benzene at two pilot stations (frequent measurements) and other monitoring stations (13 measurements per year) at 1.5 m above the ground (24 hour mean concentrations).

Pilot stations:  
Duesseldorf, Cornelius street (55 000 vehicles per day) (96 measurements in 1993),  
Essen, Hindenburg street (35 000 vehicles per day) (87 measurements in 1993).

Other stations:  
Duesseldorf-Moersbroich and Essen East (13 measurements per year each).

**Flag:** Risk Assessment

16-MAR-2004

(887)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** The (differential optical absorption spectrometer, DOAS) registered about 100 spectra per second within the wavelength range of 250-290 nm. The spectra were averaged over 1 min to provide for a sufficient signal/noise-ratio. The concentration was calculated using Lambert-Beer's law and reference spectra of pure air and pure reference substances recorded in advance. The minimum detectable concentration of DOAS was 5 µg/m<sup>3</sup>, the cross sensitivity for toluene was less than 2 %.

The VOC analyser (adsorption on Tenax, thermal desorption and cryofocussing on a capillary column, capillary GC - FID) had a cycle for measuring and analysing of 1 h with a sampling period of 30 min per cycle.

**Result:** Benzene concentrations in µg/m<sup>3</sup>:

Annual mean concentrations by means of DOAS were: 40 (1992) and 47 (1993).

Monthly average concentrations by means of DOAS varied between 31 and 60 (August and December 1992) and between 31 and 67 (June and January 1993).

Continuous measurements show the peak concentrations in January 1993 and in July 1993: in January, 2 maxima were above 150 and 6 maxima in the range 100-150; in July, 3 maxima were above 60 and 7 in the range 50-60 (readings from a diagramm).

Diurnal concentration changes were investigated by the VOC analyser on January 20 1994: The minimum was at 6 hours (8), the morning maximum at 11 hours (45), the afternoon minimum at 16 hours (159), and the evening maximum at 21 hours (55).

A comparison between DOAS and VOC analyser shows a generally agreement with some discrepancies at peak concentrations due to emission differences in the two locations of the monitoring equipment.

**Source:** German Rapporteur

**Test condition:** Monitoring at 2 sites in the historic Centre of Rome: An optical instrument (differential optical absorption spectrometer, DOAS) was used at Santa Maria Maggiore, and a VOC analyser was used at Piazza Venezia (about 1 km away behind the hill Monte Viminale).

**Flag:** Risk Assessment

16-MAR-2004

(152)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** Annual mean concentrations of four cities for the UK in 1993 are reported ( $\mu\text{g}/\text{m}^3$ ):  
Edinburgh 4.5;  
Belfast South 6.2;  
Cardiff East 9.4;  
London Bloomsbury 7.1.

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(810)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Result:** Continuation of monitoring reported in a previous report: Hanus-Illnar A, Hrabcik I: Ambient air concentrations of benzene, toluene and xylene (btx). UBA-95-124; Wien, November 1995. Monitoring at 11 sampling stations over one whole year gave the following data:  
Averages over 14 days ranged from 1.6 and 17.5  $\mu\text{g}/\text{m}^3$ . Annual averages ranged from 4.7 to 12.8  $\mu\text{g}/\text{m}^3$ . At 2 of the 11 sampling positions, the planned immission limit of 10  $\mu\text{g}/\text{m}^3$  was exceeded.

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(477)

**Type of measurement:** concentration at contaminated site

**Medium:** other: rain water

**Result:** Monitoring data show that rain water contains benzene in concentrations of 0.1 to 0.46  $\mu\text{g}/\text{l}$  in city areas of Berlin (no further details on location, time and type of measurements reported).

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(653)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** Benzene was sampled by passive absorption on Tenax TA for one week. Absorbed benzene was thermally desorbed and analysed by capillary-GC-FID with the use of external standards. The detection limit was 0.16  $\mu\text{g}/\text{m}^3$ .

**Result:** Mean benzene concentrations in 12 Swedish cities in the winter seasons 1992/1993, 1993/1994 and 1994/1995 (in  $\mu\text{g}/\text{m}^3$ ) (read-out from a diagram):

Stockholm: 6.4 / 4.7 / 3.4

Uppsala: 4.7 / 3.8 / 2.5

Joenkoepping: 4.3 / 3.5 / 2.6

Vaemamo: 6.1 / 2.8 / 2.9

Aelmhult: 4.7 / 3.8 / 2.9

Kristianstad: 4.8 / 3.1 / 2.5

Landskrona: 5.3 / 4.3 / 2.9  
Falkenberg: 4.4 / 3.3 / 2.6  
Goeteborg: 7.9 / 4.8 / 4.3  
Karlstad: 10.4 / 7.4 / 6.4  
Timra: 4.5 / 4.2 / 3.3  
Lulea: 6.2 / 5.5 / 3.5

The absorption efficiency depends upon temperature, relative humidity and wind speed. For benzene and other aromatic hydrocarbons, the repeatability is 3.5 - 5.3 %. Potential re-desorption under sampling conditions as determined by comparison with the BTX-Analyser is in the order of 2.5 % and thus within experimental error.

**Source:** German Rapporteur

**Test condition:** Samples were taken in 12 Swedish cities in winters (from October to March) in the winters 1992/1993, 1993/1994 and 1994/1995. Sampling sites were chosen to represent the highest concentrations of benzene without direct influence from point sources.

**Flag:** Risk Assessment

16-MAR-2004

(798)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** The 24 hour air samples were pooled on a 2 week basis in order to give two samples per month. Extracts of charcoal (carbon disulphide) were analysed by capillary-GC-FID.

**Result:** Two concentrations per month from May 1992 to April 1993 were reported.

Lowest concentration reported: 6.9 µg/m<sup>3</sup> (2nd half of August 1992).

Highest concentration reported: 36.0 µg/m<sup>3</sup> (2nd half of November 1992).

Distribution of concentrations (in µg/m<sup>3</sup>):

3 below 10,  
12 between 10 and 20,  
5 between 20 and 30,  
4 above 30.

In parallel, particulate matter was collected on glass fibre filters and tested for mutagenicity.

**Source:** German Rapporteur

**Test condition:** Air was sampled by absorption on charcoal tubes with an air flow of 200 ml/min. Sampling took place in a four-lane street with heavy traffic in Rome every alternate day for 24 hours from May 1992 to April 1993.

**Flag:** Risk Assessment

16-MAR-2004

(385)

**Type of measurement:** concentration at contaminated site**Medium:** air**Method:** Air samples were absorbed on Carbosieve III / carbopack B, thermally desorbed and analysed by capillary-GC (detector not reported, presumably FID).  
The detection limits (LOD) were 5 - 20 ppt depending on the species, accuracies were better than 25 % above 50 - 100 ppt and better than 50 % near the LOD.**Result:** Detailed results were reported for 2 flights on 19 July 1996 (readings from a diagram)  
(ppt / ng/m<sup>3</sup>; asl = above sea level):  
Morning flights: 160 / 520 (1000 m asl),  
80 / 260 (3000 m asl).  
Afternoon flights: 260 / 830 (1000 m asl),  
110 / 370 (3000 m asl).The afternoon flight in about 3000 m asl was reported in detail (benzene concentrations in ppt / ng/m<sup>3</sup>):  
Segment 1: 105 / 340; segment 2: 100 / 320;  
segment 3: 107 / 350.**Source:** German Rapporteur**Test condition:** An experiment evolving measurements of hydrocarbons in the atmosphere took place in Switzerland in the Mesolcina valley south of the San Bernardino pass from 18 July to 19 August 1996. Air was analysed on the ground in the town of Grono and in small aircraft cruising above the valley and the mountains surrounding the valley.  
Sampling time on the ground was 1725 s (volume: 3 l), in the aircraft 545 s (volume: 1.35 l).

16-MAR-2004

(909)

**Type of measurement:** concentration at contaminated site**Medium:** air**Method:** Air samples were collected on Carbotrap B, Carboxen 1000 and Carboxen 1001 at ambient temperature (50 ml/min for 20 min). The organic compounds were thermally desorbed and analysed by capillary-GC-PID.  
The detection limit was 0.001 ppb (= 0.003 µg/m<sup>3</sup>).**Remark:** Maximum benzene concentrations were measured in the morning rush hours between 8.00 and 9.00 (13-14 December 1995) or between midnight and 6.00 in the morning (23-24 May 1996) while on 29 June - 1 July 1997 no distinct pattern could be detected.**Result:**

Date of sampling	Number of samples	mean ± SD (ppb / µg/m <sup>3</sup> )	range
23-24 Nov. 1994	26	0.53 ± 0.11	0.37 - 0.75
13-14 Dec. 1995	36	1.13 ± 0.52	0.36 - 2.35
22-24 May 1996	35	0.75 ± 0.64	0.06 - 2.31
29 June-1 July 1997	53	0.38 ± 0.23	0.19 - 1.71
18-21 Oct. 1997	47	0.88 ± 0.56	0.18 - 3.13

**Source:** German Rapporteur**Test condition:** Sampling took place in the urban atmosphere of Yokohama at Hiyoshi from November 1994 to October 1997. For 24 - 48 hours, samples in a 1-hour cycle were automatically taken on 23-24 November 1994, 14-15 December 1995, 22-24 May 1996, 29

June - 1 July 1997 and 18-21 October 1997.

16-MAR-2004

(1290)

**Type of measurement:** concentration at contaminated site**Medium:** air

**Method:** A differential optical absorption spectrometer was used to monitor the concentrations of different inorganic and organic compounds on different wavelengths in the UV region. The measured atmospheric spectrum was divided by the pre-recorded light source reference spectrum to eliminate the wavelength dependence of the optical parts of the instrument. The quotient is divided by the fifth order polynomial which suits the broad band wavelength variations of the spectrum. The resulting spectrum (= differential spectrum) is free of the extinction effects of atmospheric particles.

The spectral interval used for the measurement of aromatics (incl. benzene) is 240 - 275 nm. The mathematical algorithm used for the determination of the concentrations of trace gases is based on the multiple correlation of the measured spectrum with a reference spectrum which is calculated from pre-recorded reference differential absorption cross-sections of the main interfering atmospheric gases in the same spectral region.

For the distance of 165 m, the minimum detectable quantity for the aromatics as provided by the manufacturer is around 0.5 ppb while the linearity is 2 ppb in the 0-250 ppb range.

**Remark:** In 1997, the passenger car fleet in Greece comprised about 60 % conventional cars and 40 % cars with catalysts and about 1 % cars with diesel engines (all diesel passenger cars were taxis that accounted for about 10 % of total mileage in Athens and Thessaloniki), whereas all heavy duty vehicles were diesel vehicles. With further introduction of catalysts in passenger cars and diesel passenger cars, the concentration of benzene will change in the atmosphere.

**Result:** The time coverage during the period of measurements was 71 % for benzene.

The daily averages of benzene concentrations vary between 1 and 6 ppb (= 3.2 - 19 µg/m<sup>3</sup>).

The frequency distribution of hourly average benzene concentrations from December 1993 to August 1994 divided into intervals of 0.5 ppbV (= 1.6 µg/m<sup>3</sup>) is as follows (ppbV / µg/m<sup>3</sup>):

< 0.5 / < 1.6:	6.08 %
0.5 - 1.0 / 1.6 - 3.2:	6.97 %
1.0 - 1.5 / 3.2 - 4.9:	11.89 %
1.5 - 2.0 / 4.9 - 6.5:	13.34 %
2.0 - 2.5 / 6.5 - 8.1:	12.52 %
2.5 - 3.0 / 8.1 - 9.7:	9.77 %
3.0 - 3.5 / 9.7 - 11.3:	9.6 %
3.5 - 4.0 / 11.3 - 13.0:	7.69 %
4.0 - 4.5 / 13.0 - 14.6:	6.75 %
4.5 - 5.0 / 14.6 - 16.2:	5.04 %
5.0 - 5.5 / 16.2 - 17.8:	3.11 %
5.5 - 6.0 / 17.8 - 19.4:	2.24 %
6.0 - 6.5 / 19.4 - 21.1:	1.52 %

6.5 - 7.0 / 21.1 - 22.7: 1.11 %  
> 7.0 / > 22.7: 2.36 %

**Source:** German Rapporteur  
**Test condition:** Measurements took place in Thessaloniki, the second largest city in Greece, from December 1993 to August 1994. The measuring site was located on the campus of the University with the two ends of the measuring path (165 m length, 50m above ground) on two different buildings.

16-MAR-2004

(639)

**Type of measurement:** concentration at contaminated site  
**Medium:** air

**Method:** Air samples on a monthly average basis were collected by passive absorption on Chromosorb-106 in stainless steel tubes following ISO/CD 16017-2 (air quality - sampling and analysis of VOC by sorbent tube/thermal desorption/capillary gas chromatography - Part 2: diffusive sampling). The samples were thermally desorbed and analysed by capillary-GC-FID. Calibration was performed by external standards; the limit of detection was estimated at lower than 0.01 µg/m<sup>3</sup> for all alkane, alkene and aromatic compounds.

**Remark:** Benzene concentrations dropped markedly after the shut-down of the waste incinerator only at sites south-east to east of the incinerator while remaining almost constant west, north-east and south of the incinerator.

**Result:** Benzene concentrations measured were in the range 5.1 - 136.9 µg/m<sup>3</sup>.  
Breakdown of monitored data in terms of monitoring site and the periods before and after the shutdown of the waste incinerator:

Sampling site	before shutdown	after shutdown
SS1 (1)	16.1	20.7
SS2 (1)	15.3	19.5
SS3 (1)	14.1	12.3
SS4 (2)	120.6	46.4
SS5 (2)	80.7	35.1
SS6 (2)	59.4	30.3
SS7 (2)	40.2	21.4
SS8 (1)	17.3	16.6
SS9 (1)	6.6	5.9

(1) Sites to the west (SS1-3), north-east (SS8) and south (SS) of the incineration plant, (2) sites to the east to south-east of the incineration plant.

**Source:** German Rapporteur  
**Test condition:** The industrial area south of the Southampton container Port Area contains a sewage treatment plant, a municipal waste incinerator and a waste recycling and processing centre. In this area, sampling took place at 9 different sites on a monthly basis from the first day to the last day of the months April, July, September and December 1996, April and August 1997. The waste incinerator was shut down in November 1996.

16-MAR-2004

(672)

**Type of measurement:** concentration at contaminated site**Medium:** ground water**Method:** Gas chromatography - mass spectrometry; further details of sampling and sample preparation not reported.**Remark:** The paper does not contain any hint whether the samples had been collected in 1986 or 1988.**Result:** Benzene was found in 4 of 11 samples at concentrations of 2 - 11 ppb from landfills that contain wastes from foundries where the following organic binder systems had been used: phenol formaldehyde, phenolic urethane, furan nobake, and phenolic ester.**Source:** German Rapporteur**Test condition:** Groundwater was analysed at four ferrous foundry waste landfills in Wisconsin containing only foundry waste. At each landfill site, three downgradient and one upgradient wells were placed in order to collect groundwater samples from different depths. Monitoring took place in two campaigns in 1986 and 1988.

16-MAR-2004

(469)

**Type of measurement:** concentration at contaminated site**Medium:** air**Method:** Passive sampling: Permeation passive samplers were placed in the flats examined. Calibration took place by exposing passive samplers to known concentrations in exposure chambers.

Active sampling: a six-channel automatic sampler was used to collect indoor air samples. The concentration were determined from known air sample volumes and the masses of benzene trapped on the absorbent.

Analysis for passive and active samplers: Desorption by carbon disulfide, capillary-GC-FID.

Detection limits ( $\mu\text{g}/\text{m}^3$ ): 0.1 (passive sampling), 0.5 (active sampling, 12 h), 1.7 (active sampling, 1 h), recovery: 95%.**Result:** Benzene concentrations in  $\mu\text{g}/\text{m}^3$ :

Flat 1:

August 1997: passive sampling. All results < 0.1  
(n = 34) active sampling (12 h): all results < 0.5.October 1997: passive sampling: 39.6 - 68.7; mean: 47.1  
(n = 30) active sampling: 24.4 - 404.4; mean: 81.6May 1998: passive sampling: all results < 0.1  
(n = 40) active sampling: 1.8 - 68.2; mean: 17.8

Flat 2:

March 1997: passive sampling: 79.5 - 88.1, mean: 82.7  
(n = 9) active sampling: 1.4 - 9.6; mean: 6.0May 1997: passive sampling: 35.6 - 43.2, mean: 39.9  
(n = 9) active sampling: 20.9 - 25.9, mean: 23.5

June 1997: passive sampling: 10.7 - 22.4, mean: 14.3  
(n = 80) active sampling: 2.2 - 22.2, mean: 6.7

March 1998: passive sampling: 23.0 - 31.4, mean: 25.6  
(n = 30) active sampling: 5.6 - 31.6, mean: 24.7

**Source:** German Rapporteur

**Test condition:** Passive samplers were placed in the flats examined in the height of the breathing zone (1,5 m above ground) for 4-5 weeks.

Flow rates for active sampling: 12 hour sampling: 20 l/min,  
1 hour sampling: 80 l/min.

Two flat were examined:

1. Downtown, 30 year old, influenced by heavy road traffic.
2. Suburb, 1 year old, influenced by moderately heavy traffic.

16-MAR-2004

(1304)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** Duplicate samples were collected using passive canisters and thermal desorption followed by GC-MSD (calibration by external standard method).

**Result:** Benzene concentrations in  $\mu\text{g}/\text{m}^3$ .

Zefun tunnel, October 18, 1997:

10-11: 54.7; 11-12: 62.8; 12-13: 75.6; 13-14: 89.5; 14-15: 77.4; 15-16: 95.3; 16-17: 118.4.

Average: 82.0; relative standard deviation: 26.0%.

Lishin tunnel, January 24, 1998:

7-9: 31.9; 9-11: 202.1; 11-13: 93.1; 13-15: 37.8; 15-17: 120.8; 17-19: 36.4.

Average: 87.0; relative standard deviation: 77.0%.

**Source:** German Rapporteur

**Test condition:** Two road tunnels in the region of Taipei were investigated: The Zefun tunnel, 200 m long, two-way. Sampling in 1-hour intervals from 10.00 to 17.00.

The Lishin tunnel, 90 m long, two one-way lanes. Sampling in 2-hour intervals from 7.00 to 19.00.

16-MAR-2004

(533)

**Type of measurement:** concentration at contaminated site

**Medium:** ground water

**Method:** Purge and trap sample preparation (head space analysis) and GLC-MS or capillary-GC-FID (only few details reported, reference to earlier papers).

**Result:** Benzene concentrations in mg /l:

Average composition of waters from different oil production platforms:

Brage: 4.5

Oseberg F: 4.6

Oseberg C: 3.7

Troll: 0.8

Daily variations of benzene concentrations from one oil production platform (Brage):

3.6 - 4.1 - 4.0 - 4.1 - 4.5  
**Source:** German Rapporteur  
**Test condition:** Water as a byproduct of offshore oil production is separated from the oil, injected into the well or discharged into the North Sea. These waters contain changing concentrations of benzene and other organic and inorganic pollutants. For benzene analyses, 10 ml samples were processed.

16-MAR-2004

(1194)

**Type of measurement:** concentration at contaminated site**Medium:** air**Method:** Air samples were sampled in canisters and analysed in accordance with USEPA Method TO-14 (US EPA 1997) by GC-MSD (no further details reported).

The limit of detection was 0.2 ppbV for benzene.

**Result:** Benzene concentrations (mg/m<sup>3</sup>; n = 62):  
1.02 - 11.16; mean: 4.85, standard deviation: 2.21.**Source:** German Rapporteur**Test condition:** Air sampling took place at Hong Kong Polytechnical University at a point 6 m above ground and 8 m away from a road with 170 000 vehicles per day passing by. The sampling period was from April 1999 to March 2000.

16-MAR-2004

(510)

**Type of measurement:** concentration at contaminated site**Medium:** air**Method:** Whole air samples were collected by personal whole air sampler units that use mass flow control to collect a constant flow into an evacuated sampling container. Adsorbent tube samples were collected using pumps at flow rates of 17 and 67 ml/min. Canister samples were analysed after pressurising with a neutral gas (calculating the dilution factor from the pressures before and after addition of the neutral gas) by cryofocussing and capillary-GC-MS (ion trap instrument) with a packed pre-column.

Adsorbent samples were analysed by thermal desorption, focussing on a trap at 27°C and capillary-GC-MS (ion trap instrument).

Benzene was quantified by both methods.

**Result:** Overall results from canisters and tubes at Sao Paulo (number of samples / mean (ppbV) / standard error of means (ppbV)):

Canisters: 34 / 2.6 / 0.4. -- Tubes: 78 / 2.5 / 1.0

Results from the above described sampling sites using canisters | tubes

(number of samples / mean (ppbV) / standard error of means (ppbV)):

(1) 6 / 3.5 / 0.5 | 16 / 0.8 / 0.2

(2) 6 / 1.4 / 0.3 | 16 / 0.8 / 0.2

(3) 6 / 1.4 / 0.1 | 12 / 1.5 / 0.1

(4) 6 / 3.5 / 0.6 | 6 / 2.9 / 0.6

(5) 4 / 0.9 / 0.1 | 4 / 0.0 / 0.0

(6) 2 / 11.0 / 0.4 | 2 / 0.4 / 0.0

The "root mean square" as a measure of precision of the two methods employed shows the following picture:  
Canister method:  
RMS ranges from 0.9 to 7.6 % per sampling point, overall 3.8 %.  
Tube method:  
RMS ranges from 10.9 to 39.7 % per sampling point, overall 26.6 %.

A comparison between the two methods shows generally lower measured concentrations in the absorbent tubes as compared to the canisters.

Results from 1998 at Sao Paulo, Brazil (mean benzene concentrations, ppbV):  
canister method: 2.6; tube method: 1.7.

**Source:**

German Rapporteur

**Test condition:**

Brazil has a car fleet of about 6 million of which 71 % use "gasohol" (containing 24 % ethanol), 22 % pure ethanol and 7 % (primarily trucks and buses) diesel fuel.  
At six monitoring sites in the region of Sao Paulo, air samples were collected for 1, 2 and 4 hours with breaks of about 20 min for sample changes. One set of canisters and 2 - 4 sets of solid adsorbent tubes were used for each sampling period:

- (1) Cerquerira Cesar: university campus near a road with heavy traffic in the morning and late afternoon.
- (2) Ibirapuera Park: city park in the middle of the metropolitan area.
- (3) Lapa: city disposal yard along an eight lane avenue with constantly heavy traffic containing many trucks.
- (4) Congonhas: public school yard in a residential / commercial area along an avenue with constantly heavy traffic containing many trucks, a petrol station across and the airport within 0.3 km distance.
- (5) Dom Pedro Park: a park in downtown Sao Paulo surrounded by 3 major avenues.
- (6) Tunel 9 de Julho: very busy automobile and bus tunnel with trucks not allowed. Sampling took place about 150 m from the entrance.

Sampling took place after 1998 (this year is referred to as that of a previous sampling period).

16-MAR-2004

(228)

**Type of measurement:** concentration at contaminated site**Medium:** air

**Method:** Particle-free air samples were collected in canisters previously evacuated with a constant flow of 10-15 ml/min for 24 hours per sample. An aliquot of the air sample was condensed by colling with liquid argon and thermally desorbed. Analysis was performed on a gas chromatograph (capillary column) equipped with a FID that was calibrated with NIST standards at different dilutions.

**Result:** Benzene concentrations (ppb):  
Ulsan downtown: 1.1 +/- 0.6  
Ulsan industrial area: 2.1 +/- 0.8

For comparison, data from another industrial site (Yochon, November 1996 - March 1997, 24 hour sampling, 21 samples) were reported: 2.1 +/- 1.0 ppb.

**Source:** German Rapporteur

**Test condition:** In the city of Ulsan (south-east coast of Koera) and industrial areas outside the city including an oil refinery, air measurements took place on June 3 to 15, 1997. The oil refinery processes about 810 000 barrels a day, and the inhabitants (about 1 million) with more than 240 000 vehicles (79 % passenger cars, 15 % trucks, 5 % busses) consume about 910 million l oil per year. At each sampling site, 5 samples each were taken.

16-MAR-2004

(807)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** Ambient air samples were collected in steel canisters with an air flow of 10-15 ml/min over 24 hours. Analyses of C4-C9 hydrocarbons (including benzene) were performed with a capillary-GC-MS system connected to a packed preconcentration trap with glass bead. An aliquot of the air sample was concentrated in the cooled precolumn (at - 196°C) and thermally desorbed onto the analytical column. The analytical precision was within 15 % at concentrations above 5 ppbC (= 0.83 ppbV for benzene). The limit of detection was reported as 0.1 ppbC, the limits of quantification in the region of 0.1 - 0.5 ppbC for all compounds investigated.

**Result:** During the monitoring period, 4-5 samples were collected per month, in all 55.

Benzene concentrations, monthly and annual mean values (ppb):

1998

August	September	October	November	December
0.8	0.9	1.1	1.2	1.3

1999

January	February	March	April	May	June	July
1.2	1.2	1.0	0.8	0.8	0.8	0.7

Annual mean: 1.0

According to the authors, the seasonal variations may be affected by the following factors: variation of VOC source strength, variation in atmospheric mixing pattern (i. e. increased vertical mixing by convection during summer) and variation in OH radical concentration and thus photochemical degradation.

**Source:** German Rapporteur

**Test condition:** The capital city of Korea, Seoul, has about 10 million inhabitants and about 2.3 million vehicles (76.2 % passenger cars, 14.3 % trucks and 9.4 % busses). Air measurements took place in the north-east of Seoul on top of a four-story building 18 m above ground 100 m away from a major road from August 1998 to July 1999 every 6 days.

16-MAR-2004

(806)

**Type of measurement:** concentration at contaminated site

**Medium:** air

**Method:** Benzene was sampled on Radiello diffusive samplers consisting of active carbon in a porous polyethylene body with a flow rate of 80 ml/min. Every measuring campaign started on Monday morning and ended on Friday evening (about 110 h). Afterwards, the samplers were desorbed with carbon disulfide and analysed for e. g. benzene by capillary-GC-FID. The uncertainty used on a 95 % confidence interval was less than 25 %. Based on replicates, the repeatability was better than 10 % (2 x standard deviation).

**Result:** Benzene concentrations, geometric means (gm) and 98 percentiles ( $\mu\text{g}/\text{m}^3$ ):

Campaign	97/41	97/48	98/04	98/13	98/24	98/40	annual
(year/week)							
average							
gm exposure	5.82	6.01	9.59	2.98	3.56	1.35	5.22
gm home	4.81	4.75	6.89	2.85	2.17	1.81	4.30
gm outdoor	3.37	2.96	4.71	2.08	2.47	1.68	2.90
98 % expos.	11.53	16.83	23.37	7.91	34.87	4.68	16.65
98 % home	15.54	15.35	20.47	6.86	14.45	10.25	14.88
98 % outdoor	6.80	7.54	9.24	5.45	6.51	4.90	6.08

**Source:** hot spot 10.97 10.59 11.29 8.04 7.36 5.40 8.94  
German Rapporteur

**Test condition:** The main source of benzene in the atmosphere is benzene from petrol-fuelled cars. In 1994, petrol contained 3.5 % benzene. In the following years, the benzene concentration was lowered, e. g. in summer 1998 from 2.0 % to 1.0 %. The project presented here is Monitoring of Atmospheric Concentrations in European Towns and Homes (MACBETH) where benzene exposure was measured in 6 European cities. In Copenhagen (250 000 inhabitants), 50 volunteers took part in a monitoring program from October 1997 to October 1998. The program comprised a questionnaire concerning possible benzene exposure from the surroundings, smoking, oil stoves, type of work etc. The volunteers were divided into two groups (A: working mainly outdoors, B: working mainly indoors). In 6 campaigns, the personal exposure of the volunteers as well as at 70 locations throughout Copenhagen (open places with out or with few benzene sources in grids of 0.5 km x 0.5 km) were measured A station in downtown Copenhagen was chosen as "hot spot" in terms of benzene exposure (Jagtvej).

16-MAR-2004

(1043)

**Type of measurement:** concentration at contaminated site**Medium:** air

**Method:** Air was sampled for 20 minutes on Tenax TA filled in glass tubes (sample volume 2 l). The samples were thermally desorbed, kryofocussed and analysed by capillary GC-FID. Calibration took place every day with solutions of 4 different known concentrations in methanol. The accuracy was below 10 %, the relative standard deviation of repeated injections of the standards below 7 %. The detection limit was 0.01 ppbv.

**Result:** Results are presented for benzene (only overall statistics for all measurements) and BTX (also differentiated in terms of measuring period, measuring site and local time). BTX comprises benzene, toluene, ethyl benzene, all 3 isomer xylenes, and 1,3,5-trimethylbenzene.

Statistics of all measurements (concentrations in ppbv)  
(benzene / BTX):

Mean value:	0.81 / 3.89
Median value:	0.58 / 2.85
Standard deviation:	0.70 / 3.18
Maximum:	4.14 / 16.85
Minimum:	< 0.01 / 0.49
Number of samples:	130 / 130

BTX statistics for sampling periods:

Sampling period:	February	May	September	October
Mean value:	3.00	6.18	3.52	4.38
Median value:	2.14	4.91	2.93	3.08
Standard deviation:	2.21	4.44	2.44	3.61
Maximum:	12.61	16.85	10.69	14.90
Minimum:	0.64	0.83	0.49	1.17
Number of samples:	53	18	26	33

BTX statistics for sampling sites:

Sampling site	1	2	3	4	5	6
Mean value:	4.10	3.35	4.84	4.55	3.38	3.05
Median value:	3.16	2.24	3.48	3.48	3.06	2.04
Standard deviation:	3.60	3.86	3.16	3.54	1.62	2.97
Maximum:	16.85	14.77	12.13	12.61	7.97	14.90
Minimum:	1.05	0.64	1.38	0.83	0.83	0.49
Number of samples:	37	11	9	20	18	40

BTX statistics for different local times:

Local time	0.00	6.00	12.00	18.00
Mean value:	3.47	3.91	3.62	3.95
Median value:	2.61	2.89	2.53	3.05
Standard deviation:	2.62	3.51	10.15	3.44
Maximum:	12.61	12.44	60.82	14.90
Minimum:	1.03	0.64	1.23	0.49
Number of samples:	29	35	36	35

**Source:** German Rapporteur

**Test condition:** An oil refinery at the Gulf of Korinthos (60 km west of Athens) was investigated in order to determine atmospheric pollution by specific hydrocarbons (e. g. benzene). At 6 different sites around the refinery (in a distance of 3 km),

air monitoring took place at different periods in 1997 (22 February - 2 March, 4 - 12 May, 14 - 21 September, 25 October - 2 November) 4 times a day (midnight, 6.00, 12.00 and 18.00 hours local time).

16-MAR-2004

(592)

**Type of measurement:** other: 15 different sites in a German state.

**Medium:** air

**Remark:** In Feb 1995 a study at 15 sites in Sachsen, Germany showed: 6.0 µg/m<sup>3</sup> annual average (Freiburg), 98% value 19 µg/m<sup>3</sup>, 2-6 µg/m<sup>3</sup> monthly average of 15 sites, 3-11 µg/m<sup>3</sup> max. daily average. Location 12 (Borna) heavy industrial area: monthly average 5 µg/m<sup>3</sup>, 98% monthly value 15 µg/m<sup>3</sup>, max daily average 9 µg/m<sup>3</sup>, max 3 hr, average 23 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(990)

**Type of measurement:** other: 3 different urban areas.

**Medium:** air

**Remark:** In 1987-1993 a study at 3 cities in Hessian area of Germany showed: 3.1-8.8 ug/m<sup>3</sup> average, 98% value 10.4-45.7 ug/m<sup>3</sup>, Hanau (Jan 1993-Dec 1993); 1.6-6.6 ug/m<sup>3</sup> average, 98% value 6.7-31.8 ug/m<sup>3</sup>, Kassel (July 1989-June 1990); 1.5-4.0 ug/m<sup>3</sup> average, 98% value 5.9-29.7 ug/m<sup>3</sup>, Wetzlar-Giessen (July 1987-June 1988).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(508)

**Type of measurement:** other: Comparison of regional and street-border measurements.

**Medium:** air

**Remark:** In 10/92-9/93 a study at Rhein, Lorrach, Grenzach-Wyhlen, Rheinfeldern showed regional mean, range and 98% value (µg/m<sup>3</sup>):  
Weil am Rhein/Lorrach 3.2, 2.2-4.9, 13;  
Grenzach-Wyhlen/Rheinfeldern 2.9, 2.0-4.4, 10.  
Street measurements annual mean, 98% value, and range of 30 minute samples (µg/m<sup>3</sup>):  
Grenzach-Wyhlen 8.7, 34, 0.0-36.2;  
Rheinfeldern 6.5, 17, 0.1-20.9.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(783)

**Type of measurement:** other: Influent and effluent of an industrial wastewater treatment plant

**Medium:** other: industrial wastewater.

**Remark:** Wastewater samples were collected from the influents to treatment systems (API separator effluents) of two refineries and at final discharge points after treatment (final effluents).

The mean influent concentration of benzene was 15.6 mg/l. The mean final effluent concentration of benzene was less than a detection limit of 6 µg/l.

The overall decrease of benzene due to wastewater treatment is greater than 99.9%.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(27)

**Type of measurement:** other: Monitoring in an inner-city street.

**Medium:** air

**Remark:** In Jan-March 1995 a study at Rheinland, Mainz-Parcusstrasse site showed a 3 month period - average 10.85 ug/m3. Max monthly average 11.97 ug/m3, max daily average 22.5 ug/m3, max 30 min average 96.11 ug/m3. Values for March 95, average 9.28 ug/m3, max daily 16.76 ug/m3, max 30 min 42.89 ug/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(658)

**Type of measurement:** other: absorption through human skin

**Medium:** other: human skin

**Result:** Absorption of benzene through the skin increases as the size of the dose increases.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(370)

**Type of measurement:** other: average and maximum immision concentration on a monthly basis

**Medium:** air

**Remark:** In Oct 94-June 95 a study at 3 locations in Aue, Bautzen andBorna (6 other sites similar range of values 3-7 µg/m3) showed: (all values µg/m3 30 minute sample) monthly max -- Borna 26.5-72.3, Bautzen 27.7-43.7, Aue 20.6-37.1; monthly average -- Borna 3.7-6.8, Bautzen 3.7-5.7, Aue 3.9-6.7.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(990)

**Type of measurement:** other: benzene concentrations depend on the density of traffic.  
**Medium:** air

**Remark:** In 1990-1993 a study in 3 sites in Bavaria showed 3.3 ug/m3 average background, 9.3 ug/m3 average traffic, 13.3 ug/m3 average heavy traffic.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (115)

**Type of measurement:** other: calculation  
**Medium:** other: not reported

**Result:** Risk Simulation software.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (866)

**Type of measurement:** other: center of a large city.  
**Medium:** air

**Remark:** In Oct 1990 - July 1991 a study at Bavaria - Northwest centre of Munich, front of BLFU showed 8.1 ug/m3 average, < 0.1 - 136.3 ug/m3 range of continuous measurements.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (111)

**Type of measurement:** other: cigarette smoke in a smoke chamber  
**Medium:** air

**Result:** Benzene measured in sidestream smoke ranged from 345-653 ug/cigarette.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (160)

**Type of measurement:** other: cigarette smoke in an smoking chamber  
**Medium:** other: cigarette smoke

**Result:** In tests with a smoking machine in a chamber to which only the sidestream smoke was lead while the mainstream smoke was vented outside an amount of 500 ug benzene/cigarette was measured. For comparison the indoor air benzene levels in anUS American tavern which are probably mainly caused by smoking were determined. They showed values of 21 and 27 ug/m3 respectively while outdoor concentrations of 8 and 6 ug/m3 were measured simultaneously.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004 (700)

**Type of measurement:** other: comarison of the Ruhr area with Berlin.

**Medium:** air

**Remark:** In 1990 a study at Nordrhein-Westfalen polluted areas situated along Rhine and Ruhr rivers showed:  
Rhein-Ruhr - 1990  
mean 3.5 µg/m<sup>3</sup>, min 1.3 µg/m<sup>3</sup>, max 9.9 µg/m<sup>3</sup>;  
Ruhrgebiet 1990 regional means µg/m<sup>3</sup>  
East 3.28, Middle 3.71, West 3.01;  
Rhein µg/m<sup>3</sup> -- Mid 3.78, South 3.91;  
Berlin average 5.1 µg/m<sup>3</sup>, range of averages 3.3-8.8 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(691)

**Type of measurement:** other: comparison of 11 urban sites and 1 rural site

**Medium:** air

**Method:** Organic compounds were absorbed on Chrompack, thermally desorbed, cryogenically trapped and analysed by gas chromatography (detector not reported, presumably FID).

**Remark:** Benzene was one of 26 compounds monitored in all.  
Emissions from the transport sector in 1996 were estimated as follows (data from the UK National Atmospheric Emissions Inventory, NAEI):  
Road transport from diesel powered vehicles: 1 810 t.  
Road transport from petrol powered vehicles: 22 690 t.  
Petrol evaporation: 1 820 t.  
Overall benzene emissions: 26 320 t.

**Result:** Mean benzene concentrations and number of valid hourly data points (N) in the ratified data set (ppb / µg/m<sup>3</sup> / N):  
Birmingham: 1.02 / 3.30 / 7525  
Cardiff: 1.21 / 3.92 / 8266  
Leeds: 1.04 / 3.37 / 8240  
Edinburgh: 0.70 / 2.27 / 8332  
Harwell: 0.38 / 1.23 / 7448  
Bristol: 1.22 / 3.95 / 8234  
London Eltham: 1.06 / 3.43 / 8281  
Middlesbrough: 1.01 / 3.27 / 8191  
Belfast: 0.92 / 2.98 / 8169  
Southampton: 1.92 / 6.22 / 8114  
UC London: 1.87 / 6.06 / 8298  
Liverpool: 0.89 / 2.88 / 7981

**Source:** German Rapporteur

**Test condition:** The National Hydrocarbon Network consisted of 12 sites in 1996. Samples were collected hourly for 24 hours a day during 1996. The sites contained an automatic equipment consisting of a thermo-desorption/cryogenic trapping system, and a gas chromatograph.

16-MAR-2004

(294)

**Type of measurement:** other: comparison of 15 different cities of different size.  
**Medium:** air

**Remark:** In 1988-1994 a study at 15 cities in Bavaria (some duplication with TUEV 1987/1988 reported data in HEDSET) showed 1 ug/m<sup>3</sup> (Furth i Wald) - 14.4 ug/m<sup>3</sup> (Munich) range of average.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (117)

**Type of measurement:** other: comparison of 3 different cities of different size.  
**Medium:** air

**Remark:** In 1992/1993 a study at 16 cities in Bavaria showed 2.65 (Hof)-18.32 ug/m<sup>3</sup> (Munich) range of average for 4 day samples - 1992; and 2.9 (Augsburg) - 14.9 ug/m<sup>3</sup> (Munich) range of average for 4-6 day samples.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (114)

**Type of measurement:** other: comparison of 3 regions within 1 Federal state.  
**Medium:** air

**Remark:** In 1993 a study at Baden-Wurttemberg 3 regions identified for benzene measurements showed regional mean, range and 98% value (ug/m<sup>3</sup>):  
Rhein/Loerrach 3.2, 2.2-4.9, 13;  
Grenzach-Wyhlen/Rheinfelden 2.9, 2.0-4.4, 10;  
Ulm, Neu-Ulm, Umgebung 3.0, 2.0-5.8, 12.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (1184)

**Type of measurement:** other: comparison of 6 city areas in East Germany.  
**Medium:** air

**Remark:** In 1993 a study at 6 sites in East Germany showed (all values ug/m<sup>3</sup>):  
Weissenfels: average 3.3, range 2.1-5.9, 98% value 15.  
Naumburg: average 2.1, range 1.3-5.2, 98% value 10.  
Zeititz: average 3.4, range 2.1-7.0, 98% value 17.  
Hohenmolsen: average 2.2, range 1.7-4.3, 98% value 10.  
Theissen-Bornitz-Profen: av. 2.8, range 2.0-4.1, 98% value 13.  
Lutzen: average 2.1, range 1.7-2.5, 98% value 12.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (657)

**Type of measurement:** other: comparison of 6 sites in Eastern Germany.

**Medium:** air

**Remark:** In Oct 1992-Sept 1993 a study at 6 sites in East Germany showed (all values  $\mu\text{g}/\text{m}^3$ ):  
Weissenfels: average 3.3, range 2.1-5.9, 98% value 15.  
Naumburg: average 2.1, range 1.3-5.2, 98% value 10.  
Zeititz: average 3.4, range 2.1-7.0, 98% value 17.  
Hohemolsen: average 2.2, range 1.7,4.3, 98% value 10.  
Theissen-Bornitz-Profen: av. 2.8, range 2.0-4.1, 98% value 13.  
Lutzen: average 2.1, range 1.7-2.5, 98% value 12.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(778)

**Type of measurement:** other: comparison of a large urban area and a medium-sized town.

**Medium:** air

**Remark:** In 1991 a study at Berlin and Brandenburg (about 50 km west of Berlin) 12 sampling locations showed :  
6.9  $\mu\text{g}/\text{m}^3$  annual average, 1.6-14.5  $\mu\text{g}/\text{m}^3$  range, 30 l/15 min samples.

Concentration average ( $\mu\text{g}/\text{m}^3$ ):  
1988-6.1, 1989-5.4, 1990-5.1, 1991-7.0.

Note 1991 was the first year that the former East Berlin was included in measurements.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(1172)

**Type of measurement:** other: comparison of a medium-sized town and a semi-rural area.

**Medium:** air

**Remark:** In 4/90-3/91 a study at Pforzheim-Muehlacker showed:  
Pforzeim range of yearly averages 1.7 to 5.5  $\mu\text{g}/\text{m}^3$ ,  
98% value 6-24  $\mu\text{g}/\text{m}^3$ ;  
Muehlacker range of yearly values 1.6-3.4  $\mu\text{g}/\text{m}^3$ ,  
98% value 6-14  $\mu\text{g}/\text{m}^3$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(780)

**Type of measurement:** other: comparison of an industrial town and a university town.

**Medium:** air

**Remark:** In 10/90-10/91 a study at Reutlingen/Tuebingen showed:  
Reutlingen range of yearly averages 1.32 to 3.5  $\mu\text{g}/\text{m}^3$ ,  
98% value 4.37-16.23  $\mu\text{g}/\text{m}^3$ ;  
Tuebingen range of yearly averages 1.25-3.95  $\mu\text{g}/\text{m}^3$ ,  
98% value 4.16-15.36  $\mu\text{g}/\text{m}^3$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(781)

**Type of measurement:** other: comparison of areas with and without an industrial influence

**Medium:** air

**Method:** Average samples of ambient air over 24 hours were absorbed on active charcoal (2 l/min). The carbon disulphide extract of the charcoal was analysed by capillary-GC-FID. The limit of detection was reported as 10 - 50 ng/m<sup>3</sup> depending upon the analysed compound but not reported for benzene specifically.

**Remark:** The monitoring program had the aim to accompany legislation on the reduction of VOC emissions.

**Result:** Average concentration s at the 3 locations were as follows (µg/m<sup>3</sup>):

Name of location	average concentration ± standard deviation	number of samples
Hujbergen	1.9 ± 2.0	543
Moerdijk	2.1 ± 2.6	541
Vredepeel (03/1991-12/1996)	1.6 ± 1.5	483

A downward trend (± 95 % confidence interval) for the sum of all VOCs is reported for the period of 1991 - 1997:

Hujbergen: - 4.4 ± 2.2 % / year

Moerdijk: - 0.2 ± 3.4 % / year

Vredepeel: - 3.9 ± 2.4 % / year (03/1991 - 12/1996)

For comparison, benzene monitoring data from other stations were reported:

Kollumerwaard (near the North Sea):

monitoring since June 1994, 1-hour samples every 2 weeks, results divided up into winter (November - February) and summer (May - August) and marine / continental wind sections (sampling period: 06/1994 - 07/1995) (µg/m<sup>3</sup>):

marine, winter: 0.52; continent, winter: 1.96;

marine, summer: 0.46; continent, summer: 0.72.

RIVM monitoring network (20 sample per year, distinctin between regional, urban and street stations), sampling period: 01 - 12/1993, concentrations in µg/m<sup>3</sup>:

Regional: 1.1

City: 2.8

Street: 4.5

**Source:** German Rapporteur

**Test condition:** At 3 locations in the south of the Netherlands (Noord-Brabant), VOCs were monitored from March 1991 to February 1997.

16-MAR-2004

(1124)

**Type of measurement:** other: comparison of benzene in gasoline and exhaust gases.  
**Medium:** other: liquid gasoline, gasoline vapour, exhaust gas.

**Remark:** The mean benzene concentration in automobile exhaust was reported to be 8.8% of the total volatile organic compound emissions.  
Gasoline vapour typically contains 1.1% benzene.

Volatile organic compounds present in refinery air samples (process area) are typically 2.2% benzene.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(229) (237)

**Type of measurement:** other: comparison of contaminated site and background  
**Medium:** air

**Method:** Automated GC-FID analytical system

**Result:**

Period	Four-lane street	University building
	Benzene concentrations (ppbV / $\mu\text{g}/\text{m}^3$ )	
Feb. 1994	9.7 / 31.4	1.6 / 5.2
May 1994	8.2 / 26.6	1.5 / 4.9
August 1994	5.0 / 16.2	0.8 / 2.6
December 1994	4.4 / 14.3	1.1 / 3.6
March 1995	3.8 / 12.3	1.4 / 4.5
Mean	6.2 / 20.1	1.3 / 4.2

**Source:** German Rapporteur

**Test condition:** Monitoring at two places in the center of Copenhagen:  
1.: 3 m above a four-lane street canyon with some 26 000 vehicles per day passing by.  
2.: on the roof of a university building (20 m above street level) some hundred m away from the street described under 1.

Measurements for five 3-week periods from February 1994 to March 1995.

**Flag:** Risk Assessment

16-MAR-2004

(475)

**Type of measurement:** other: comparison of emissions and immissions.

**Medium:** air

**Remark:** In 7/91-7/92 a study at Kehl/Offenburg showed range of yearly averages 2.2 to 4.8  $\mu\text{g}/\text{m}^3$ ;  
1990 annual emissions < 1 t/a.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(782)

**Type of measurement:** other: comparison of exhaled air with and without smokers in the same flat

**Medium:** other: exhaled air

**Result:** Non-smokers who live with smokers exhale air with higher concentrations of benzene (3.6 to 4.2 ug/m<sup>3</sup>) than non-smokers who do not live with smokers (1.5 to 2 ug/m<sup>3</sup>).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(1221)

**Type of measurement:** other: comparison of flats near petrol stations and away from petrol stations

**Medium:** air

**Result:** All benzene concentrations in µg/m<sup>3</sup>.  
Benzene concentrations in 32 flats near petrol stations:

	inside / outside the flats	
arithmetic mean:	10.2	/ 9.3
standard deviation:	4.0	/ 3.4
maximum:	22.4	/ 20.3
median (50 %):	9.9	/ 9.1

Benzene concentrations in 7 reference flats:  
inside / outside the flats

arithmetic mean:	5.6	/ 4.8
standard deviation:	1.5	/ 1.3
maximum:	8.0	/ 7.1
median (50 %):	5.7	/ 5.0

The benzene emissions in the flats near petrol stations were independent of the distance from the petrol stations (range: 10 - 40 m), altitude above the street level (ground floor to 4th upper floor) and the traffic in the streets besides these flats (low - medium - heavy traffic; no further information reported).

**Source:** German Rapporteur

**Test condition:** Measurements in the air inside and outside flats took place near petrol stations and as reference flats without petrol stations or other commercial activities associated with hydrocarbon emissions in November 1993:

**Flag:** Risk Assessment

16-MAR-2004

(503)

**Type of measurement:** other: comparison of indoor and ambient air

**Medium:** air

**Method:** Air samples were absorbed on charcoal at the same time inside and outside the homes. After desorption with carbon disulphide, the organic compounds were analysed by capillary-GC (detector not reported, presumably FID). The desorption efficiency for benzene was between 92 % (for toluene) and 100 % (for ethylbenzene). Field blanks (i. e. not used charcoal tubes) were analysed as well.

**Remark:** This study was performed to evaluate differences of air

pollutants inside and outside homes in streets with high and low traffic intensity.

**Result:** Benzene was not detected in the field blanks. The limit of detection was not reported for benzene, but for toluene (0.05  $\mu\text{g}/\text{m}^3$ ) and m- / p-xylene (0.19  $\mu\text{g}/\text{m}^3$ ).

The results were combined for both measuring campaigns ( $\mu\text{g}/\text{m}^3$ ):

High traffic homes:  
Mean: 5.7; median: 5.2; minimum: 2.8; maximum: 10.6;  
Low traffic homes:  
Mean: 3.0; median: 3.1; minimum: 1.6; maximum: 4.9.

**Source:** German Rapporteur

**Test condition:** Sampling (24 hour average measurements) of indoor air (in the living rooms) and outdoor air (in front of the homes) took place at 36 homes in the centre of Amsterdam (equal numbers of homes with high and low traffic density were measured). Homes were selected so that parking lots, garages or service stations were not in the vicinity of the homes (i. e. not within 30 m). Other selection criteria were: no smoking in the home, living-room at street-side, home front close to the street (i. e. no wide pavement or bicycle-lane). Samples were taken in 2 campaigns in January-February and March-April 1995 on 19 days and 14 days respectively.

16-MAR-2004

(360)

**Type of measurement:** other: comparison of polluted and background sites  
**Medium:** air

**Country:** Belgium, Hungary, Latvia

**Method:** Air samples were passed through carbon absorbers (15 l in polluted areas and 30 l in rural areas) and analysed by capillary-GC-FID (most likely, detector not reported, only an integrator for peak integration was mentioned).

**Remark:** Air samples and pine tree needle samples were collected in parallel in order to assess the possibility of monitoring pine needles in terms of air pollution.

**Result:** Average air concentrations (averages of all 4 seasons sampled) were reported ( $\mu\text{g}/\text{m}^3$ ):

Belgium:  
3.07 (busy road), 1.78 (petrol station), 0.85 (remote area).  
Hungary:  
2.30 (busy road), 2.52 (petrol station), 1.27 (remote area).  
Latvia:  
7.80 (busy road), 7.87 (petrol station), 1.05 (remote area).

**Source:** German Rapporteur

**Test condition:** Sampling took place from August 1994 to August 1995 in 3 different countries along a busy road, near a petrol station and in a rural site simultaneously within 2 weeks of 4 consecutive seasons. In each sampling period, 4 air samples (1 hour each) per sampling site were taken, i. e. in all 16 air samples per sampling site.

02-AUG-2002

(614)

**Type of measurement:** other: comparison of polluted and background sites  
**Medium:** biota

**Method:** Needles of pine trees were collected, sent in air-tight vessels to the laboratory and analysed by extraction with dichloromethane and GC-MSD.

**Remark:** Air samples and pine tree needle samples were collected in parallel in order to assess the possibility of monitoring pine needles in terms of air pollution.

**Result:** In all needle samples, the benzene concentration was at or below the detection limit of about 40 µg/kg dw.

**Source:** German Rapporteur

**Test condition:** Sampling took place from August 1994 to August 1995 in 3 different countries along a busy road, near a petrol station and in a rural site simultaneously within 2 weeks of 4 consecutive seasons. In each sampling period, needle samples from 5 different pine trees per sampling site were taken, i. e. in all 20 needle samples per sampling site.

16-MAR-2004

(614)

**Type of measurement:** other: comparison of polluted areas within a city with a non polluted area

**Medium:** air

**Method:** Air was passed through magnesium perchlorate and alkali asbestos to absorb water vapour and carbon dioxide and then through a polymer bead cooled by liquid nitrogen to absorb volatile organic compounds. The organic compounds were desorbed, kryofocussed, and analysed by capillary-GC-MS and capillary-GC-FID.

**Remark:** Seasonal variations were reported in terms of total VOC immission concentrations. Maximum immission concentrations were measured in January or November, and minimum immission concentrations in May.

**Result:** Average concentrations were reported for each of the 5 sampling sites

mean / standard deviation / range; µg/m<sup>3</sup>):

A (roadside): 38.5 / 29.1 / 18.2 - 134.6

B (downtown): 43.8 / 20.1 / 23.4 - 103.9

C (industrial area): 22.3 / 9.1 / 5.7 - 53.5

D (residential area): 20.6 / 8.7 / 7.7 - 47.7

E (reference area outside Changchun): 9.4 / 6.0 / 2.9 - 27.5

As a comparison, data from other city centers were reported (ppbv / µg/m<sup>3</sup>):

Grenoble (May 1995): 0.5 - 3.0 / 1.6 - 9.7

Bangkok (1995): 5.6 / 18.1

Manila (1995): 3.9 / 12.6

**Source:** German Rapporteur

**Test condition:** Monitoring took place in 4 places within the town of Changchun in North-East china and one place south-east of Changchung from September 1997 to July 1998. Sampling took

place on 5 consecutive days every 2 months three times a day (9-13 September, 5-9 November, 2-7 January, 5-9 March, 1-5 July).

In all, 450 samples were collected in this time.

16-MAR-2004

(698)

**Type of measurement:** other: comparison of suburban areas.

**Medium:** air

**Remark:** In 1/92-2/93 a study at Mannheim/Heidelberg showed regional mean, range and 98% value ( $\mu\text{g}/\text{m}^3$ ):  
Mannheim/Ladenburg 3.6, 2.0-6.4, 15.5;  
Weinheim/Schriesheim 3.1, 2.2-4.9, 12.9;  
Dossenheim/Heidelberg 3.5, 2.3-5.9, 16.7;  
Sandhausen/Leimen/Wiesloch 3.2, 2.3-4.6, 13.7.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(1182)

**Type of measurement:** other: comparison of the Ruhr area and Hamburg.

**Medium:** air

**Remark:** In 1989 a study at Nordrhein-Westfalen polluted areas situated along Rhine and Ruhr rivers showed:  
Rhein-Ruhr - 1989 mean  $4.8 \mu\text{g}/\text{m}^3$  min 1.8, max 9.7;  
Ruhrgebiet 1989 regional means  $\mu\text{g}/\text{m}^3$  - East 4.9, Middle 5.2, West 4.0;  
Rhein  $\mu\text{g}/\text{m}^3$  - Mid 5.3, South 5.0;  
Hamburg (1986-1987) - average  $8.2 \mu\text{g}/\text{m}^3$ , range 6.9-10.6  $\mu\text{g}/\text{m}^3$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(690)

**Type of measurement:** other: comparison of two-way road, crossing and car park

**Medium:** air

**Method:** Sorption of the hydrocarbons on active carbon, desorption by carbon disulfide, GC-analysis (detector not reported).

**Result:** Benzene concentrations ( $\mu\text{g}/\text{m}^3$ ):  
Preliminary measurements (1992; 24-hour mean values):  
Large road (medium traffic): 10 - 33  
Well-ventilated road with high traffic: 10 - 26  
Narrow road with high traffic: 13 - 36  
Narrow road with medium traffic: 8 - 37  
Ventilated road with high traffic: 11 - 35

Monitoring in 1993 (March - December; 24-hour mean values):  
(max./min./medium/standard deviation)

Two-way road: 41 / 2 / 14 / 6.5  
crossing: 64 / 15 / 32.5 / 8.4

Underground car park

(3-hour mean values; readings from diagrammes):

Date (1993)	Minimum (time)	Maximum (time)
October 18:	< 25 (before 7 h)	350 (10 h)
October 19:	< 25 (before 7 h)	280 (19 h)

October 20: < 25 (before 7 h) 280 (10 and 19 h).

**Source:** German Rapporteur

**Test condition:** Preliminary measurements in 1992 at five different places. Systematic monitoring at a two-way road used by a bus line, a crossing of 6 roads (in both places at 1.7-2 m above the ground and 1.5 m from the street border) and an underground car park (at 1.5 m above the ground) in 1993 (March - December).

**Flag:** Risk Assessment  
16-MAR-2004 (249)

**Type of measurement:** other: comparison of urban and rural areas

**Medium:** air

**Method:** General mentioning of "online gaschromatographic techniques" without reporting further experimental details.

**Remark:** Comprehensive survey of several field campaigns in and around Munich from 1993 to 1997.

**Result:** At one place in downtown Munich (1), the following benzene concentrations were obtained in the time 12 -26 August 1993 (ppbV /  $\mu\text{g}/\text{m}^3$ ):  
median: 5.7 / 18.5; mean value: 7.0 / 22.7;  
maximum: 32.1 / 104  
number of data above detection limit: 603 (LOD not reported).

At the same place (1), the following benzene concentrations were obtained in the time 8 April - 2 May 1994 (ppbV /  $\mu\text{g}/\text{m}^3$ ):  
median: 4.6 / 14.9  
mean value: 5.3 / 17.2  
maximum: 25.0 / 81.0  
number of data above limit of detection: 1112.

Compilation of mean values, maximum values and number of data above the limit of detection (see also test conditions for the allocation of numbers in brackets):

Locations/ dates above	mean values (ppbV / $\mu\text{g}/\text{m}^3$ )	maximum values	number of data
			LOD
(1)			
12.-26.08.1993	3.0 / 9.7	14.9 / 48.3	603
30.09.-10.10.1993	2.3 / 7.5	18.7 / 60.6	231
18.10.-29.10.1993	3.0 / 9.7	8.3 / 26.9	778
08.04.-02.05.1994	2.8 / 9.1	13.8 / 44.7	1111
28.04.-24.05.1994	0.9 / 2.9	13.6 / 44.1	929
(2)			
26.05.-27.07.1995	0.5 / 1.6	4.3 / 13.9	1629
28.09.-10.10.1995	0.4 / 1.3	2.3 / 7.5	180
(3)			
01.04.-18.04.1993	0.7 / 2.3	2.4 / 7.8	568
04/1993-05/1995	0.5 / 1.6	9.3 / 30.1	9744
29.05.-10.08.1993	0.5 / 1.6	5.8 / 18.8	1760
27.07.-26.09.1995	0.1 / 0.3	0.6 / 1.9	316
04.07.-25.07.1996	0.3 / 1.0	6.0 / 19.4	712
06.05.-19.05.1997	0.1 / 0.3	0.6 / 1.9	863

Data from other publications on urban areas around the world (ppbV /  $\mu\text{g}/\text{m}^3$ ):

London 1992: 3.0 / 9.7

Athens 1994 (2 sites):

(1) 9.5 / 30.8 (median), 61.2 / 198 (maximum),

(2) 1.6 / 5.2 (median), 23.5 / 76.1 (maximum).

**Source:** German Rapporteur

**Test condition:** Monitoring places were as follows:

(1) 3 places in downtown Munich,

(2) 1 place in the south-east of Munich,

(3) 1 place south-west of Munich (distance about 50 km, 775 m asl) and 3 places to the north to east of Munich

(distances about 30 km, 563 m asl or lower).

16-MAR-2004

(925)

**Type of measurement:** other: comparison of urban and semi-rural areas.

**Medium:** air

**Remark:**

In 1992 a study at Baden-Wurttemberg 6 regions identified for Benzene measurements showed: regional mean, range, and 98% value ( $\mu\text{g}/\text{m}^3$ )

Mannheim/Ladenburg 3.6, 2.0-6.4, 15.5;

Weinheim/Schriesheim 3.1, 2.2-4.9, 12.9;

Dossenheim/Heidelberg 3.5, 2.3-5.9, 16.7;

Sandhausen/Leimen/Wiesloch 3.2, 2.3-4.6, 13.7;

Offenburg 3.2, 2.3-4.2, 13.6;

Kehl 2.8, 2.2-4.8, 13.7.

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

29-JUL-2002

(1183)

**Type of measurement:** other: comparison of urban area and remote area.

**Medium:** air

**Result:**

Prior to 1991 a study at Frankfurt City and Schauinsland Schwartzwald (mountainous area) showed: Indoor 15  $\mu\text{g}/\text{m}^3$ , outdoor 19  $\mu\text{g}/\text{m}^3$ ; indoor/outdoor ratio = 0.8; Frankfurt city 19  $\mu\text{g}/\text{m}^3$ , residential area, 11  $\mu\text{g}/\text{m}^3$ , Schauinsland 3  $\mu\text{g}/\text{m}^3$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Flag:**

Risk Assessment

16-MAR-2004

(802)

**Type of measurement:** other: comparison of urban, suburban, rural and remote regions.

**Medium:** air

**Method:**

Benzene concentrations were determined with a Proton-Transfer-Reaction Mass Spectrometer by reacting  $\text{H}_3\text{O}^+$ -ions with benzene molecules in the gas samples collected on-line at the sampling sites as protonated species with  $m/e = 79$  and the ration 79/80 for information about the number of C-atoms in the molecule from the percentage of C-13 (1.1 % natural abundance).

**Remark:**

Comparison of traffic emissions (automobiles) and biomass burning (during dry season) in terms of their contribution

to total benzene emissions.

**Result:** The benzene concentrations in pmol benzene/mol air /  
ng/m<sup>3</sup>:

- (1) averages: 3300 / 10700 (morning),  
1100 / 3600 (11.00 - 16.00).
- (2) average: 270 / 880; 25-percentile: 150 / 490;  
75-percentile: 330 / 1070 (11.00 - 16.00).
- (3) Wet season: average: 83 / 270; 25-percentile: 57 / 185;  
75-percentile: 110 / 360;  
Dry season: average: 150 / 490; 25-percentile: 100 /  
325;  
75-percentile: 550.
- (4) Dry season: average: 31 / 100; 25-percentile: 17 / 55;  
75-percentile: 43 / 140.

**Source:** German Rapporteur

**Test condition:** Air samples were taken at 4 stations:

- (1) downtown Caracas,
- (2) University campus (20 km from downtown Caracas),
- (3) woodland savanna in the plains north of the Orinoco  
river,
- (4) grassland savanna south of the Orinoco river.

The times of sampling were:

- (1) 4 days in February 2000,
- (2) December 1999 and February 2000,
- (3) 24 September - 17 October 1999 (wet season) and  
18 March - 9 April 2000 (dry season),
- (4) 20 January - 4 February 2000 (dry season).

16-MAR-2004

(522)

**Type of measurement:** other: comparison of different regions of a Federal state  
in 1992.

**Medium:** air

**Remark:** In 1992 a study at Nordrhein-Westfalen polluted areas  
situated along Rhine and Ruhr rivers showed:  
Rhein-Ruhr 1992 mean 3.01 µg/m<sup>3</sup>, min 1.51, max 5.59;  
Ruhrgebiet regional means µg/m<sup>3</sup>  
East 2.44, Middle 3.15, West 2.84;  
Rhein Mid 3.3 µg/m<sup>3</sup>, South 3.56 µg/m<sup>3</sup>;  
LRP averages 2.16 µg/m<sup>3</sup>, range 1.26-5.29 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(692)

**Type of measurement:** other: comparison of different regions of a Federal state  
in 1993.

**Medium:** air

**Remark:** In 1993 a study at Nordrhein-Westfalen polluted areas  
situated along Rhine and Ruhr rivers showed:  
Rhein-Ruhr 1993 mean 2.75 µg/m<sup>3</sup>, min 1.2, max 5.06;  
Ruhrgebiet regional means (µg/m<sup>3</sup>)  
East 2.6, Middle 3.05, West 2.25;  
Duesseldorf 3.0 µg/m<sup>3</sup>, Koeln 2.92 µg/m<sup>3</sup>;  
LRP average 5.18 µg/m<sup>3</sup>, range 3.24-10.9 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn

29-JUL-2002 German Rapporteur (659)

**Type of measurement:** other: dermal exposure to benzene  
**Medium:** other: human skin

**Result:** Flux rate 400 ug/cm<sup>2</sup>-hour was used to establish dose for dermal exposure to benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004 (473)

**Type of measurement:** other: different parts of an urban area.

**Medium:** air

**Remark:** In Aug 1988, Jan, 1989 and June, 1989 a study at 7 locations in Wiesbaden, Hessian area of Germany showed:  
3-27 ug/m<sup>3</sup> range of daytime sample (n=3-10) averages, range of values <0.5-46 ug/m<sup>3</sup>; 3 gas stations range of average 10-26 ug/m<sup>3</sup>, range of values 2-56 ug/m<sup>3</sup>, 10 day samples; 1 M from nozzle, 2-3 min sample range 1020-5615 ug/m<sup>3</sup>, N=7, average 2720 ug/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002 (502)

**Type of measurement:** other: domestic and industrial landfills

**Medium:** other: gas from landfills

**Result:** Benzene was detected in only one of the three municipal waste landfills (4.2 mg/m<sup>3</sup>).

In the 3 industrial waste landfills, the following concentrations were measured:

114 mg/m<sup>3</sup>, about 6 mg/m<sup>3</sup>, 23 mg/m<sup>3</sup>.

**Source:** German Rapporteur

**Test condition:** Gas samples were taken from six different landfills 3 of which contain only domestic refuse and the other 3 at least partially industrial wastes from a depth of at least 1 m. Organic compounds were sampled by sorption on Tenax or Porapak Q and analysed by GC-MS.

**Flag:** Risk Assessment

16-MAR-2004 (1303)

**Type of measurement:** other: exposure of the general public

**Medium:** air

**Result:** The arithmetic mean levels of benzene to which the general public is exposed are calculated to range from 8.6 ug/m<sup>3</sup> (2.7 parts per billion, ppb) to 10.9 ug/m<sup>3</sup> (3.4 ppb). For the worker sub-populations, mean exposures are generally below 3200 ug/m<sup>3</sup> and therefore the cancer risk MOSs are above 1. Gathering information, and/or more testing is indicated for a few groups of workers.

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(182)

**Type of measurement:** other: hazardous and sanitary landfills**Medium:** other: leachate

**Result:** The following concentrations were measured ( $\mu\text{g/l}$ ):

Hazardous landfills					Sanitary landfills			
A1	A2	A3	A4	A5	B1	B2	B3	B4
150	50	380	20	1180	178	572	1.1	1.2

**Source:** German Rapporteur**Test condition:** The leachates from 5 hazardous landfills and 4 sanitary landfills were analysed for volatile organic compounds (e. g. benzene) by headspace-GC-MS and spiking with standard solutions.**Flag:** Risk Assessment

16-MAR-2004

(386)

**Type of measurement:** other: heavily polluted industrial area in Germany.**Medium:** air

**Remark:** In 1980-1988 a study at towns and cities in Nordrhein-Westfalen showed:  
range of average near cokers 10 to 30  $\mu\text{g}/\text{m}^3$ ; range of 1987-88 average for 62 town and city locations 3.6-17  $\mu\text{g}/\text{m}^3$ ; range of average near various industries 1.5-17.8  $\mu\text{g}/\text{m}^3$ , aromatics plant average 7.9  $\mu\text{g}/\text{m}^3$ , range 4.4-15.5  $\mu\text{g}/\text{m}^3$ ; range during smog period 20.9-85.6  $\mu\text{g}/\text{m}^3$ .

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(693)

**Type of measurement:** other: industrial area and residential area in a small town.**Medium:** air

**Remark:** In Nov. 1988 - Aug. 1989 a study at Burghausen (Eastern industrial area) Burgkirchen (Western residential area) showed 3.5 - 7.0  $\mu\text{g}/\text{m}^3$  average for area (0.83-2.51 ppb); 3.07-11.06 ppb (9.8 - 35.4  $\mu\text{g}/\text{m}^3$ ) 98% for Western area (Max 49 ppb); 4.24-31.33 ppb (13.5 - 100  $\mu\text{g}/\text{m}^3$ ) 98% for Eastern area (Max 32.4 ppb).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(112)

**Type of measurement:** other: long term changes in the center of London**Medium:** air**Method:** Sorption onto Tenax-GC/TA by active sampling, thermal desorption, gas chromatographic analysis equippec with a FID.

**Result:** Benzene concentrations dropped from 1979 to 1991:  
1979: 30.9  $\mu\text{g}/\text{m}^3$  (9.5 ppb),  
1982: 29.9  $\mu\text{g}/\text{m}^3$  (9.2 ppb),  
1983: 32.9  $\mu\text{g}/\text{m}^3$  (10.2 ppb),  
1986: 20.9  $\mu\text{g}/\text{m}^3$  (6.5 ppb),  
1987: 22.9  $\mu\text{g}/\text{m}^3$  (7.1 ppb),

1991: 13.0 µg/m<sup>3</sup> (4.0 ppb),  
1992: 11.0 µg/m<sup>3</sup> (3.4 ppb).

This means a reduction of 64 % in 1992 in comparison to 1979 despite an increase in traffic in the same period.

The benzene content in premium petrol was as follows:  
1980: 2.9 %, 1984: 2.7 %, 1986: 3.6 %.

**Source:** German Rapporteur  
**Test condition:** The monitoring place was at Exhibition Road in the center of London with about 1500 vehicles per hour.  
The monitoring period was 1979, 1982-1983, 1986-1987, and 1991-1992 in the summer time (i. e. April - September) at day time (i. e. 8.00 to 16.00).  
In 1979 and 1982-1983, a packed GC column was used,  
In 1986-1987 and 1991-1992, a capillary column was used.  
**Flag:** Risk Assessment  
16-MAR-2004 (357)

**Type of measurement:** other: long-term trend in the Netherlands  
**Medium:** air

**Result:** Urban benzene concentrations in the atmosphere had a downward trend.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
16-MAR-2004 (4)

**Type of measurement:** other: measurements in a school and outside the same school.

**Medium:** air

**Remark:** In 1993 a study at Munich (duplication with HEDSET data by DEKRA and TUEV) showed measurements inside school and classroom 9 µg/m<sup>3</sup> in classroom, 10 µg/m<sup>3</sup> in school yard, 14 µg/m<sup>3</sup> outside near street. Mobile measurements ranged from 10-21 µg/m<sup>3</sup> (Nov-Dec 1993).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
29-JUL-2002 (116)

**Type of measurement:** other: measurements near streets.

**Medium:** air

**Remark:** In 1994/1995 a study in Bavaria (147 values near roads) showed street benzene concentrations range from < 1 - 39 µg/m<sup>3</sup>; 57 (of 147) values > 10 µg/m<sup>3</sup>; only 3 (of 147) values > 20 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
29-JUL-2002 (118)

**Type of measurement:** other: monitoring in a large urban area.

**Medium:** air

**Result:** In 1993/1994 a study at Berlin - 18 locations with Benzene values showed: range 1.8-14 µg/m<sup>3</sup>, average 7.47 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(104)

**Type of measurement:** other: monitoring in a large urban area.

**Medium:** air

**Remark:** In 1990 study at 11 sites in West Berlin showed 5.1 µg/m<sup>3</sup> annual average, 3.3-8.8 µg/m<sup>3</sup> range.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(103)

**Type of measurement:** other: monitoring in a large urban area.

**Medium:** air

**Remark:** In 1989 a study at 11 sites in West Berlin showed 5.4 µg/m<sup>3</sup> annual average 2.6-10.3 µg/m<sup>3</sup> range. Highest values in winter. Decrease in values from previous years.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(102)

**Type of measurement:** other: monitoring in down town street canyons.

**Medium:** air

**Result:** The following benzene concentrations were reported without mentioning time and place of measurement and refer only to "down town street canyons":  
Annual average: 15 - 35 µg/m<sup>3</sup>,  
98 percentile: 46 µg/m<sup>3</sup>,  
30-min maximum: 110 µg/m<sup>3</sup>.

Semi-continuous measurements (30 min sampling) were reported for the following days (place of monitoring: down town Frankfurt/Main; results in µg/m<sup>3</sup>):

05.12.1991: 60  
14.01.1992: 64  
16.01.1992: 50,3  
21.02.1992: 103,6  
02.03.1992: 34,2  
06.03.1992: 81  
09.03.1992: 64,5  
10.03.1992: 59,8

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(975)

**Type of measurement:** other: occupational exposure to benzene

**Medium:** other: human skin

**Result:** A wide range of dermal transfer rates for benzene are identified.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(728)

**Type of measurement:** other: occupational exposure to mechanics.

**Medium:** other: not reported.

**Result:** Dermal contact may be a significant source of exposure for automobile mechanics.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

16-MAR-2004

(654)

**Type of measurement:** other: packed food

**Medium:** other: microwave-heat-susceptor food packaging

**Result:** In 1989, benzene was found among the volatile compounds of 8 from 11 different microwave susceptors packaged with 10 different US American food products in concentrations between < 1.6 and 34 ng/cm<sup>2</sup> susceptor test strip.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(756)

**Type of measurement:** other: potential exposures of adults to benzene.

**Medium:** air

**Result:** Time-activity patterns based upon surveys showed that adult smokers were in-transit for 74 min./day and outdoors for 37 min./day. Adult non-smokers were in-transit for 108 min./day and outdoors for 67 min./day. All adults spent 3 min./day at petrol stations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(26)

**Type of measurement:** other: review of benzene immisions in Germany

**Medium:** air

**Result:** A review is given on the amounts of benzene used for different industrial uses and the benzene emissions from these uses as well as on the distribution, fate and occurrence in the environment.

The latest data on ambient air in this paper are:  
Germany, January - April 1996:  
8 µg/m<sup>3</sup> (Germany as a whole),  
9 µg/m<sup>3</sup> (former West Germany),  
7 µg/m<sup>3</sup> (former East Germany),  
maximum: 183 µg/m<sup>3</sup>.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
16-MAR-2004 (124)

**Type of measurement:** other: river flowing through a large city.

**Medium:** surface water

**Remark:** Llobregata River, Barcelona, Spain.  
Sampling period September 1989 to March 1990.  
Mean concentration of benzene 0.76 µg/l,  
median concentration of benzene 0.64 µg/l,  
range of benzene concentrations 0.05 to 1.80 µg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
29-JUL-2002 (453)

**Type of measurement:** other: rural and urban areas in Switzerland

**Medium:** air

**Method:** Passive sampling over 2-week periods on activated carbon,  
elution of the activated carbon with carbon disulfide, and  
capillary gas chromatography (detector not reported).

**Result:** The following benzene concentrations (µg/m<sup>3</sup>; averages over  
the whole monitoring period) were found (n = number of  
2-week samples):

Site	n	average (+/- standard deviation)
Basel	13	1.8 (+/- 0.9)
Genève	13	3.9 (+/- 1.2)
Zurich	5	6.0 (+/- 1.4)
Lugano	13	3.1 (+/- 0.9)
Aarau	13	2.0 (+/- 1.0)
Wald	13	1.1 (+/- 0.7)
Payerne	13	1.4 (+/- 1.2)
Montana	13	0.9 (+/- 0.5)
Davos	13	0.8 (+/- 0.5)

Temporal variations of benzene concentrations showed a  
generally increasing tendency from September to December.

**Source:** German Rapporteur

**Test condition:** Monitoring took place in the following places:  
1. Aarau (small town, 25 m above ground in the town center),  
2. Basel (large city, 3 m above ground in the city center),  
3. Davos (small town, 3 m above ground outside the town),  
4. Genève (large town, 3 m above ground in the city center),  
5. Montana (remote area, 3 m above ground),  
6. Wald (rural, 3 m above ground outside the village),  
7. Payerne (rural, 2.5 m above ground outside the village),  
8. Zurich (large city, 3 m above ground in the city center).  
The monitoring period was 7th June - 6th December 1993 at  
the locations 1-7 and 4th October - 6th December 1993 at  
location 8.

The samplers were covered by shelters made of PVC in order  
to minimise the effect of wind on the sampling.

**Flag:** Risk Assessment  
16-MAR-2004 (790)

**Type of measurement:** other: rural sites

**Medium:** air

**Method:** The air collected in steel canisters was analysed by capillary-GC-FID with cryofocussing. The detection limit was 0.1 ppbC (= 0.017 ppmV for benzene with 6 C-atoms in the molecule) with a reproducibility of 30 %.

**Result:** Benzene concentrations in ppbC  
(mean / median / SD / maximum / minimum / n):

1. Centreville, Alabama  
Autumn 0.87 / 0.81 / 0.39 / 1.72 / 0.29 / 15  
Winter 1.36 / 1.32 / 0.26 / 1.83 / 0.97 / 13  
Spring 0.79 / 0.65 / 0.33 / 1.48 / 0.42 / 11  
Summer 0.61 / 0.54 / 0.23 / 0.98 / 0.33 / 10
2. Oak Grove, Mississippi  
Autumn 0.78 / 0.67 / 0.34 / 1.55 / 0.33 / 18  
Winter 1.36 / 1.29 / 0.41 / 2.30 / 0.69 / 15  
Spring 0.90 / 0.97 / 0.32 / 1.39 / 0.32 / 16  
Summer 0.69 / 0.69 / 0.24 / 1.05 / 0.17 / 12
3. Yorkville, Georgia  
Autumn 0.98 / 0.89 / 0.51 / 2.35 / 0.40 / 18  
Winter 1.79 / 1.65 / 0.99 / 4.46 / 0.97 / 13  
Spring 0.92 / 0.77 / 0.38 / 1.94 / 0.57 / 13  
Summer 1.22 / 0.91 / 0.93 / 3.57 / 0.41 / 10
4. Candor, North Carolina  
Autumn 12.77 / 12.86 / 8.35 / 24.67 / 2.14 / 12  
Winter 1.59 / 1.47 / 0.53 / 2.73 / 0.97 / 15  
Spring 1.00 / 0.87 / 0.40 / 1.83 / 0.45 / 16  
Summer 0.90 / 0.85 / 0.36 / 1.52 / 0.58 / 6

**Source:** German Rapporteur

**Test condition:** Four SOS-SCION sites (Southern Oxidants Study - Southeastern Consortium: Intermediate Oxidant Network) were selected for a monitoring program lasting from September 1992 to October 1993. There were 3-6 sampling days per month during which a 6 l air sample each was sampled in a stainless steel canister within 1 hour (12 - 13 hours local time).  
The sites were chosen to fulfill the following requirements: no large sources of SO<sub>2</sub>, nitrogen oxides within 20 - 40 km, no industrial complexes within 10 - 20 km, no city with > 50.000 inhabitants within 60 km.

16-MAR-2004

(464)

**Type of measurement:** other: suburban area.

**Medium:** air

**Remark:** In Jan-Dec 1994 a study at Frankfurt-Griesheim showed 5ug/m<sup>3</sup> annual average.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(501)

**Type of measurement:** other: time activity pattern**Medium:** other: not reported**Result:** Time activity pattern information.**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

29-JUL-2002

(712)

**Type of measurement:** other: tobacco smoke**Medium:** air**Result:** Smoking was found to be the largest anthropogenic source of background human exposure to benzene.**Source:** Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

29-JUL-2002

(484)

**Type of measurement:** other: trend analysis for selected urban areas in Germany.**Medium:** air**Remark:** Benzene concentrations for selected stations in urban areas / cities in 1995 - 1998 [ $\mu\text{g}/\text{m}^3$ ]:

1995 1996 1997 1998

Berlin	6,0	6,4	5,2	3,6
Leipzig		2,8	3,4	3,0
Karlsruhe	3,1	2,7	3,2	2,8
Magdeburg	3,3	3,9	2,7	2,0
Wiesbaden	11,8	11,0	10,0	

**Source:** German Rapporteur

29-JUL-2002

(1187)

**Type of measurement:** other: trend measurements in residential areas.**Medium:** air**Remark:** Trend Measurements for Ambient Air Concentrations in Residential Areas of Frankfurt am Main and Offenbach (Annual Averages):Year Air concentration [ $\mu\text{g}/\text{m}^3$ ]

1982 37

1983 17

1984 10

1985 11

1986 11

1987 10

1988 11

1989 12

1990 10.5

1991 10

1992 7.5

1993 5.5

1994 5.3

**Source:** German Rapporteur**Flag:** Risk Assessment

16-MAR-2004

(1175)

**Type of measurement:** other: two medium-sized towns adjacent to each other.  
**Medium:** air

**Remark:** In 10/90-10/91 a study at Heilbronn/Neckarsulm showed:  
Heilbronn range of yearly averages 1.77 to 5.56 µg/m<sup>3</sup>,  
98% value 6.32-18.98 µg/m<sup>3</sup>;  
Neckarsulm range of yearly averages 1.66-3.27 µg/m<sup>3</sup>,  
98% value 6.87-18.05 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(779)

**Type of measurement:** other: urban area  
**Medium:** air

**Remark:** In 1990-1991 a study at 22 locations in Munich, Bavaria  
showed 6.8 - 47.3 ug/m<sup>3</sup> range of average street  
measurements; and 20.8 - 179.5 ug/m<sup>3</sup> range of maximum  
values.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(113)

**Type of measurement:** other: urban area and surroundings.  
**Medium:** air

**Remark:** In Apr 1993-Mar 1994 a study at Ulm, Neu-Ulm, and  
surrounding areas showed: 3.1 µg/m<sup>3</sup> annual average -  
parking lot, range 0-14.6 µg/m<sup>3</sup>, 98% = 8.0 µg/m<sup>3</sup>; 8.4-12  
µg/m<sup>3</sup> annual average - traffic, range 0.1-51.5 µg/m<sup>3</sup>. Ulm,  
Neu-Ulm, Umgebung: average 3 µg/m<sup>3</sup>, range of average  
values 2.0-5.8 µg/m<sup>3</sup>, 98% value 12 µg/m<sup>3</sup>, range 6-28 µg/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(1185)

**Type of measurement:** other: urban area plus surroundings.  
**Medium:** air

**Remark:** In Jan-Dec 1991 a study at Untermain-West, Hessian are of  
Germany showed: 4.8 ug/m<sup>3</sup> average value per sq km. 2.6-8.2  
ug/m<sup>3</sup> range; 98% value-max 41.5 ug/m<sup>3</sup>, min 8.3 ug/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(505)

**Type of measurement:** other: urban area plus surroundings.  
**Medium:** air

**Remark:** In Jan-Dec 1992 a study at Untermain-Ost, Hessian area of  
Germany showed: 5.5 ug/m<sup>3</sup> average value per sq km,  
2.5-13.1ug/m<sup>3</sup> range; 98% value-max 69 ug/m<sup>3</sup>, min 7.5 ug/m<sup>3</sup>.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(506)

**Type of measurement:** other: urban area.**Medium:** air**Remark:** In 1993/94 a study at 8 locations in Strasbourg (border between France and Germany) showed 2.5-21 ug/m3 annual average. All averages in 5.8 ug/m3 range with one exception, Blvd. Wilson (21).**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(404)

**Type of measurement:** other: variability of benzene exposure**Medium:** other: not reported.**Result:** The assumed geometric standard deviations for benzene exposures were 2.5, 3 and 3.5 for low, moderate and high variability exposures.**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

29-JUL-2002

(644)

### 3.2.2 Field Studies

**Type of measurement:** other: in situ bioremediation**Media:** petroleum-contaminated aquifer**Method:**

Site description:

Soil and groundwater (flow velocity 0.7 cm/day), contaminated with petroleum hydrocarbons from a leaking gasoline storage tank. The groundwater had been anaerobic for at least a decade, and laboratory studies had demonstrated the presence of anaerobic bacteria capable of degrading fuel hydrocarbons. The supply of electron acceptors was limiting.

Process description:

One extraction well (4.5 l/min) and three injection wells (1.5 l/min) were installed in the aquifer. Three investigations were conducted, using the following concentrations of nitrate and sulphate:

Trial 1	15 mg/l NO3	15 mg/l SO4
Trial 2	45-55 mg/l NO3	70-80 mg/l SO4
Trial 3	85-125 mg/l NO3	70-100 mg/l

The concentrations of the injected electron acceptors were increased from one trial to the next to slowly build-up the proper microbial population. Each trial lasted 4-5 weeks with a break of 7 months and 3 months between trials. The entire study lasted 15 months from start to finish.

**Remark:** Results are presented as concentration contour plots, with no tabulation or quantation of findings.

Benzene concentrations went through a cycle of attenuation and rebound during the study. The authors suggest that this was due to dissolution of trapped NAPL below the water table or influx of benzene from areas outside the immediate remediation zone. Benzene degradation was stimulated close to the injection well by nitrate and sulphate, but only towards the

end of the 15 month treatment cycle.  
**Reliability:** (2) valid with restrictions  
 07-APR-2004

(267)

### 3.3.1 Transport between Environmental Compartments

**Type:** volatility  
**Media:** other  
**Method:** other  
**Year:** 1998

**Method:** GC-head space auto sampler

**Remark:** The effect of ionic strength on Henry's law constants of volatile organic compounds was evaluated in this research using a gas chromatograph-headspace auto sampler system. Stock sol'n @ approx. 90 ppm prep'd by 24 hr mixing in gdH<sub>2</sub>O. Final dilutions of stock sol'ns achieved approx. 500 ppb in varying concentrations of salt sol'n by spiking 1L vol of each salt sol'n w/ 5 mL of both benzene and toluene stock sol'n. Increasing volumes of each conc of spiked salt sol'n were dispensed into 8 (per salt concentration) 22 mL headspace vials to achieve different headspace to liquid vol ratios. Samples were equilibrated in the headspace auto-sampler @ 20 degree C and analyzed by GC.

The experimental results also showed that the ionic strength greater than 0.2M can lead to at least a 10% increase in Henry's constants, which in turn favors the partitioning of volatile organic compounds into the air phase. Typical sea water was simulated with 36% sodium chloride and tested from 15 degree C to 45 degree C. Henry's law constants for the studied volatile organic compounds in the typical sea water are about 30% higher than that in distilled water.

**Result:** Dimensionless Henry's Law Constants (Hc) At Different Concentrations Of Sodium Chloride

NaCl (M)	Benzene	
	Hc	R
0	0.180	0.9997
0.1	0.186	0.9998
0.3	0.208	0.9998
0.5	0.227	0.9999
0.7	0.250	0.9997
1.0	0.284	0.9998
Salting out coefficient, ks, L/mol	0.202	0.9992

Dimensionless Henry's Law Constants (Hc) At Different Temperatures in 36 % NaCl

Temperature	Benzene	
	Hc	R
15	0.184	0.9983
20	0.238	0.9996
25	0.287	0.9995
30	0.329	0.9997

	35	0.387	0.9999
	40	0.464	0.9996
	45	0.523	0.9999
<b>Source:</b>	Exxon Biomedical Sciences, East Millstone, NJ, USA Exxon Chemical Europe Inc. Bruxelles EXXON Biomedical Sciences East Millstone, NJ German Rapporteur		
<b>Reliability:</b>	(2) valid with restrictions		
29-JUL-2002			(882)
<b>Type:</b>	other: Transport and fate of benzene in unsaturated, nonisothermal, salty porous soil.		
<b>Media:</b>	other: soil		
<b>Method:</b>	other		
<b>Year:</b>	1999		
<b>Remark:</b>	<p>Simultaneous transport in soil of heat, water, potassium chloride, and benzene was studied experimentally and numerically. A laboratory experiment permitted observation of temperature, water content, chloride concentration and benzene concentration distributions in soil. A numerical model based upon newly developed transport theory was used to simulate the observed data. Transport of benzene in soils was simulated numerically under isothermal and nonisothermal conditions. Simulated results for benzene were compared with experimental data. Experiments were conducted in sealed aluminum columns (0.05-m I.D. and 0.20-m length) with sterilized salinized unsaturated Fayette soil. The soil had initial water content of 0.22 m<sup>3</sup> /m<sup>3</sup> and initial inorganic solute concentration of 0.20 mol/kg. Benzene was injected at one end of each soil column (top end) to provide 143 g/m<sup>3</sup> benzene in the upper 0.01 m portion. The isothermal soil columns were set in an incubator at 29 degree C. The nonisothermal experiment had upper and lower boundary temperatures of 34 and 21 degree C, respectively. Isothermal and nonisothermal experiments each lasted for seven days. A numerical model based upon heat and mass transfer theory was used to predict transient soil temperature, water content, and inorganic and organic chemical distributions. Both predicted and measured final temperatures showed linear distributions. In the nonisothermal study, water moved from the hot region toward the cold region, and inorganic solute accumulated in the hot region. Benzene redistributed along the soil column for both isothermal and nonisothermal conditions. Total concentration of benzene in the cold region under nonisothermal conditions was larger than the concentration of benzene in the hot region. On the other hand, the concentration of total benzene was approximately uniform along the soil column under isothermal conditions. For isothermal conditions, the benzene concentration by volume in the three phases followed the order: liquid&gt;solid&gt;gas. The results of this study indicated that transport models need to include the effect of temperature and temperature gradient to describe the movement of volatile chemicals in soils.</p>		
<b>Source:</b>	EXXON Biomedical Sciences East Millstone, NJ German Rapporteur		

**Reliability:** (2) valid with restrictions (809)  
 16-MAR-2004

**Type:** adsorption  
**Media:** water - soil  
**Method:** other  
**Year:** 1998

**Remark:** A four stage procedure was followed to evaluate benzene leachability from 6 soils consisting of 1) benzene dosing of each soil column, 2) drainage, 3) unsaturated water flow applied to soil columns & 4) 10 d benzene analysis of leachate. Each soil column was slowly saturated from the effluent end w/ 2% CaSO4 prior to dosing, drained, and then dosed w/ benzene, which was also supplied from the effluent opening. Simulated Kd for each soil were calculated using % organic carbon for each soil and a Koc value of 83 L/kg (Hasset, 1983) and compared to benzene effluent measurement. Benzene concentration in 4 of the soils leachates did not differ appreciably (approx 300 mg/L) although % organic carbon % clay, and estimated Kd are significantly different.

These results are expected to be due to the high infiltration flow rate (2.68 mL/hr) of the CaSO4 sol'n to the soils resulting in shorter contact time between the two phases, minimizing the mass tranfer of benzene to water and extending the time necessary to reach concentration equilibrium between the aqueous and solid phases. More extensive leachability of benzene was expected to have occurred in the Mitrousi soil due to the considerably lower aqueous velocity (0.65m/hr), and in the Larisa soil due to the denser soil packing, which promoted faster equilibrium of the benzene with the aqueous phase by elimination of large void pockets.

**Result:** Measured and Estimated Results of Benzene Leachability in Six Greek Soils

Soil	%Sand	%Silt	%Clay	Kd	Benzene (ppm) Leachate
Bariko	14.7	66.7	18.7	4.3	300
Zacharato	33.6	26.4	40	0.9	300
Mitrousi	2.5	32.9	64.6	0.9	700
Larisa	38.3	43.4	18.2	0.7	700
Terpylos	34.9	19.5	45.6	1.0	300
Griva	47.8	34.4	17.9	0.8	300

**Source:** Exxon Chemical Europe Inc. Bruxelles  
 EXXON Biomedical Sciences East Millstone, NJ  
 German Rapporteur

**Reliability:** (4) not assignable (400)  
 16-MAR-2004

**Type:** adsorption  
**Media:** other: air-plant  
**Method:** other: Measurements with seven different plant species  
**Year:** 1993

**Result:** Benzene was found in concentrations between 0.15 and 0.30 ug/g leaf dry wt. in leaves of *Cotoneaster dammeri* and in concentrations  $\leq 0.01$  ug/g leaf dry wt. in leaves of the other six species.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Uptake of benzene by plants was tested with samples from *Cotoneaster dammeri*, *Pinus mugo* Turra, *Pinus nigra* Arnold, *Pseudotsuga menziesii* (Mirb.) Franco, *Juniperus media* Van Melle, *Juniperus chinensis* L. and *Chamaecaparis lawsoniana* (Murr.) Parrl growing within a distance of about 1000 m along the same road with very dense traffic in the city of Gent/Belgium. Sampling was carried out in a height of about 1 m in March 1992. Benzene concentrations were determined in the air and in the plant leaves by GC-MS.

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (613)

**Type:** adsorption  
**Media:** soil - air  
**Method:** other  
**Year:** 1978

**Remark:** A study of soil samples near a benzene-using facility showed higher benzene concentrations in the soil than in the air, indicating a potential for benzene adsorption on soil, if the soil is continuously exposed to the chemical.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (742) (827)

**Type:** adsorption  
**Media:** soil - air  
**Method:** other: determination of the mass transfer by inverse gas chromatography  
**Year:** 1993

**Result:** At a temperature of 30 degree C a sorption coefficient K for benzene at the soil-air interface of 3.01 ml/g was determined. The sorption of the test substance from the gas phase decreased with increasing water content of the soil.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Soil columns were directly tested in a gas chromatograph. The test substances were injected after the dilution of saturated vapour with ambient laboratory air. Soil sorption characteristics: clay and silt fraction of an US American soil (1.2% organic matter content; 52% relative humidity (= 1.68% (w/w) water content)); temperature range: 30-50 degree C.

**Reliability:** (2) valid with restrictions

16-MAR-2004

(1086)

**Type:** adsorption  
**Media:** soil - air  
**Method:** other: experimental  
**Year:** 1984

**Method:** After the exposure of the soil to the vapours, the soil was extracted with acetone / hexane (1:1). Benzene was analysed by a spectrophotometer.

The recovery of benzene was > 97 %.

**Result:** Dry Woodburn soil adsorbed up to 29 mg benzene per g soil.

At 50 % and 90 % relative humidity, the measurements were not performed up to the full vapour pressure of benzene.

At  $p/p(0) = 0.6$ , the following results were obtained:

At 0 % relative humidity, the sorption was 9.5 mg per g soil,

at 50 % relative humidity, the sorption was 8 mg per g soil,

at 90 % relative humidity, the sorption was 2 mg per g soil.

**Source:** German Rapporteur

**Test condition:** Adsorption tests were performed in a dynamic-equilibrium sorption apparatus at a constant temperature using a Woodburn soil (60/80 mesh fraction) containing 1.9% organic matter, 9% sand, 68% silt and 21% clay. The soil samples were dried at 140°C for 48 hours and stored in vacuum desiccators.

Benzene vapours and water vapours were added by passing nitrogen over sand containing benzene and teflon tubing pieces mixed with water. Afterwards, the gas streams containing benzene and water were combined with a third gas stream in order to achieve a constant-volume gas flow.

The benzene concentration was determined by extracting the vapour at the exit of the mixing chamber with a gas impinger filled with hexane.

The humidity of the air was determined by water adsorption of magnesium perchlorate as a function of gas flow and trapping time.

The gas stream was directed into a rotating flask containing the soil sample.

**Test substance:** reagent-grade (Aldrich Chemical Co.), used as received.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

16-MAR-2004

(197)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other

**Result:** Adsorption to bottom sediments of rivers or lakes is not expected to occur to any significant degree because its volatility favours release to the atmosphere.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable

16-MAR-2004

(1281)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: see reference  
**Year:** 1983

**Result:** Benzene will be expected to exhibit very high to high mobility in soil and therefore may leach to groundwater. Koc: woodburn silt loam= 31.7-143.

**Source:** German Rapporteur  
 REPSOL PETROLEO, S.A. MADRID

**Reliability:** (2) valid with restrictions  
 16-MAR-2004 (984) (1105)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: calculation from water solubility

**Method:** Using the equations of Kenaga E.E. & Goring C.A.I. In: Aquatic Toxicology ASTM STP707. Edited by J.C. Eaton et al. American Society for Testing and Materials, 1980, soil sorption coefficients Koc were predicted for compounds (e. g. benzene) whose water solubilities were known.

**Result:** The soil adsorption coefficient was calculated for benzene to be 71 from a given water solubility of 1.78 mg/l, indicating that benzene is moderately to highly mobile. An experimental soil adsorption coefficient value of 83 was reported by Kenaga & Goring, 1980. According to a report in HSDB, 1993, this study was conducted in woodburn silt loam. Other studies have reported Koc values in this type of soil of 31 (Chiou C.T. et al. Envir. Sci. Technol. 17, 227-231, 1983 cited in HSDB, 1993) and 31.7-143 (Sabljic A. J. Agric. Fd Chem. 32, 243-246, 1984 cited in HSDB, 1993). Reliability: 2 (valid with restrictions)

**Source:** Accepted calculation method  
 Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 16-MAR-2004 (611)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: adsorption tests with radioactive labelled test substance on aquifer material

**Result:** The sorptive characteristics of benzene to two groundwater aquifer solids were studied. Log Koc values were between 2.09 and 3.01 (Koc = 123-1023) being slightly dependent of the adsorbent mass.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Experiments were carried out in capped glass centrifuge tubes with two US American groundwater aquifer materials of following characteristics:

sand	90.0%	70.4%
silt	8.0%	24.0%

clay	2.0%	5.6%
organic matter	4.4%	2.2%
pH	3.8	5.5

3 different adsorbent masses (1.0, 5.0, 10.0 g) were tested with 3 concentrations of test substance (0.078, 0.777, 7.77 mg/l) at a temperature of 20 degree C. The substance concentration was determined in the water phase by LSC.

**Test substance:** 14C labelled commercial product  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 16-MAR-2004 (1176)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: determination of adsorption isotherms with pond sediments

**Result:** For benzene, a sorption coefficient Koc related to the organic matter content of the sediment of 83 was found.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The adsorption isotherms were taken from batch tests in capped centrifuge tubes at 25 +/- 1 degree C with substance concentrations of 10-50% of the water solubility. Equilibrium conditions were achieved after 4-8 h of shaking. Analysis was carried out in the water phase. Sorbed substance concentrations were computed by difference based on the total substance amount added. Substance concentrations were determined gaschromatographically. Description of the two sediments: coarse silt fraction with an organic matter content of 2.78 and 3.27% respectively.

**Test substance:** commercial product (spectrograde, purity not given)  
**Reliability:** (2) valid with restrictions  
 16-MAR-2004 (601)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: semi-empirical method  
**Year:** 1981

**Remark:** Combination of theoretical considerations with measured parameters.

**Result:** For benzene, a sorption coefficient Koc of 60 was determined related to the organic matter content of a variety of US American soils.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Soil sorption characteristics (e.g. organic matter content, cation exchange capacity) of a variety of US American soils and measured n-octanol/water partition coefficient were combined to calculate Koc values.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 16-MAR-2004 (602)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: determination of adsorption isotherms

**Result:** For benzene, a soil sorption coefficient Koc related to the organic matter content of the soil of 18.2 (log Koc = 1.26) was measured.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The soil-solution mixtures were equilibrated for 24 h at 20 degree C in capped centrifuge tubes. The determination of substance concentration was carried out gaschromatographically in the water phase. Losses by volatilization were avoided by sampling through the septum of the caps. The substance amounts were corrected by the airspace of the tubes under consideration of air volume and Henry's Law Constant. Soil characteristics: 1.9% organic matter, 68% silt, 21% clay, 9% sand, cation-exchange capacity: 14 meqv/100 g soil.

**Test substance:** commercial product (purity not given)

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
16-MAR-2004 (196)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: calculation from molecular topology and quantitative structure-activity relationship

**Remark:** Accepted calculation method  
Calculation on the basis of first-order molecular connectivity

**Result:** For benzene, a soil sorption coefficient Koc related to the organic matter content of soils of 123 (log Koc = 2.09) was calculated.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (984)

**Type:** adsorption  
**Media:** water - soil  
**Method:** other: determination of distribution coefficients on three aquifer materials  
**Year:** 1992

**Remark:** Koc values were calculated from distribution coefficients assuming a linear relationship with the n-octanol/water partition coefficient

**Result:** Dependent on the organic matter content of the aquifer material (given in brackets) following Koc values were calculated for benzene: 759 (0.007 %), 30 (0.025%) and 42 (0.015%). The sorption of benzene appeared to increase drastically with decreasing content of organic matter.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Distribution coefficients were determined in a stainless

steel flow-through system with three different aquifer materials described as follows:

coarse sand	84%	90%	63%
fine sand	15%	8%	34%
silt	0%	0%	2%
clay	1%	2%	1%
organic matter	0.007%	0.025%	0.015%

The test substance was applied to the test columns after solution in groundwater from a Danish drinking water plant at an average influent concentration of 39 ug/l. Effluent concentrations were measured gaschromatographically.

Evaporative losses were avoided by saturating the gas phase with test substance vapour. The temperature was 10 degree C. commercial product (analytical grade, purity not given)

**Test substance:****Reliability:****Flag:**

16-MAR-2004

(2) valid with restrictions

Risk Assessment

(664)

**Type:**

adsorption

**Media:**

water - soil

**Method:**

other: determination of distribution coefficients in a batch system

**Year:**

1992

**Result:**

The distribution coefficients Kd for benzene measured at 20 different Danish aquifer materials were between 0.04 and 0.23 ml/g dependent on the content of organic matter. By dividing through the organic matter content of the aquifer material Koc values in a range between about 100 and 900 were calculated.

**Source:**

Deutsche Shell Chemie GmbH Eschborn

German Rapporteur

**Test condition:**

14C-labelled benzene in concentrations of 100, 200 and 400 ug/l solved in groundwater from a Danish drinking water plant was applied to 20 different Danish aquifer materials in capped tubes. The samples were equilibrated for 96 h at room temperature and the water phase then analysed by scintillation counting. Aquifer material characteristics: 2-94% coarse sand, 3-94% fine sand, 0-4% silt, 0-4% clay, 0.006-0.213% organic matter.

**Test substance:**

commercial product (purity not given)

**Reliability:**

(2) valid with restrictions

**Flag:**

Risk Assessment

16-MAR-2004

(665)

**Type:**

volatility

**Media:**

soil - air

**Method:**

other

**Remark:**

The half-lives of volatilization, without water evaporation, of benzene uniformly distributed at a rate of 1 kg/ha to 1 and 10 cm in soil with an organic carbon content of 1.25% were 7.2 and 38.4 days respectively.

**Source:**

BP Chemicals Ltd LONDON

German Rapporteur

**Reliability:**

(4) not assignable

16-MAR-2004

(530) (589)

**Type:** volatility  
**Media:** soil - air  
**Method:** other

**Remark:** A model was developed to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank. It was estimated that most (67%) of the benzene in the gasoline would volatilize from the soil within 17 months. Of the remaining benzene, 29% would be expected to leach to groundwater and 3% would remain in the soil.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (55) (1165)

**Type:** volatility  
**Media:** soil - air  
**Method:** other

**Remark:** Based on benzene's vapour pressure of 95.2 mmHg at 25°C (Riddick J.A. et al. Organic Solvents: Physical Properties and Methods of Purification. Techniques of Chemistry. 4th Edition. Wiley-Interscience, 1986 cited in HSDB, 1993), volatilization from soil surfaces and other surfaces is expected to be rapid. Benzene which does not evaporate will be highly-very highly mobile in soil and may leach to groundwater.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (530) (1084)

**Type:** volatility  
**Media:** water - air  
**Method:** other: calculation based on thermodynamic considerations

**Result:** The half-life for evaporation of benzene from a 1 m thick still water column was 4.81 hours at 25°C and 5.03 hours at 10°C by thermodynamic calculations. The residence half-time for well-mixed water was 37 minutes.

**Source:** BP Chemicals Ltd LONDON;  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (720) (827)

**Type:** volatility  
**Media:** water - air  
**Method:** other

**Remark:** Based on a reported Henry's Law constant of  $5.3 \times 10^{-3}$  atm-m<sup>3</sup>/mole (Hine J. & Mookerjee P.K. J. org. Chem. 40, 292-298, 1975), the half-life for volatilization from a model river 1 m deep, flowing at 1 m/second and with a wind velocity of 3 m/second was 2.7 hours at 20°C. The equations of Lyman W.J. et al. were used (Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p. 15-9 to 15-31. McGraw-Hill, New York, 1982).

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (530) (1084)

**Type:** volatility  
**Media:** water - air  
**Method:** other: see reference  
**Year:** 1982

**Result:** The estimated half-life for volatilization of benzene from a model river is 2.7 hr at 20 deg C based on a reported Henry's Law Constant of  $5.3E-3$  atm-cu m/mol.

**Source:** German Rapporteur  
REPSOL PETROLEO, S.A. MADRID

**Reliability:** (4) not assignable  
16-MAR-2004 (1125)

**Type:** volatility  
**Media:** water - air  
**Method:** other: experiments in a wind-wave tank  
**Year:** 1983

**Result:** The mass transfer coefficients of benzene at the water-air interface were 11.4-34 cm/h dependent of the wind velocity. The volatilization is of first-order kinetics. For a wind speed of 7.09 m/s a half-life of 5.2 h can be calculated.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The wind-wave tank was 6 m long, 0.61 m deep and 0.60 m wide with wind velocities of 5.96-13.2 m/s at a temperature of 20.7 degree C. The testing period was > 50 h so that an approximate 10fold change of solute concentration would occur. The solute concentration was monitored with gas chromatography.

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (721)

**Type:** volatility  
**Media:** water - air  
**Method:** other: calculated  
**Year:** 1996

**Method:** Calculation of volatilisation of benzene from flowing river water as a function of depth and flow velocity of river and temperature of river water at a low wind velocity (0.25 m/s): the transfer rate of benzene across the water/air interface is determined by the Henry's law constant and the mass transfer coefficients within the liquid and gas phases respectively.

In case of volatilisation from water the initial concentration in air is assumed  $c = 0$ .

**Result:** Winter (water temperature: 5°C, wind velocity: 0.25 m/s)  
 Half-life of benzene (in days) at water flow velocities of

	0.032 m/s	0.316 m/s	3.16 m/s
Depth of river (m)			
10 m	68.2	21.8	7.13
3.2 m	12.2	3.93	1.32
1 m	2.18	0.713	0.249
0.3 m	0.393	0.132	0.049
0.1 m	0.071	0.025	0.010

Summer (water temperature: 25°C, wind velocity: 0.25 m/s)  
 Half-life of benzene (in days) at water flow velocities of

	0.032 m/s	0.316 m/s	3.16 m/s
Depth of river (m)			
10 m	42.3	13.5	4.39
3.2 m	7.55	2.42	0.803
1 m	1.35	0.439	0.150
0.3 m	0.242	0.080	0.029
0.1 m	0.044	0.015	0.006

For a river with a depth of 3.2 m, the half-life distance at low wind velocities (0.25 m/s) varies from 20.6 km or 33.3 km (25°C or 5°C, flow velocity 0.032 m/s) to 220 km or 359 km (25°C or 5°C, flow velocity 3.16 m/s).

For a stream with a depth of 0.32 m, the half-life distance at low wind velocities (0.25 m/s) varies from 0.66 km or 1.05 km (25°C or 5°C, flow velocity 0.032 m/s) to 7.9 km or 13.4 km (25°C or 5°C, flow velocity 3.16 m/s).

So, the half-life distance of benzene is most strongly influenced by the depth of the flowing surface water and least strongly by the change in temperature (here: 5°C and 25°C).

The calculation method appears to be valid and gives results very relevant for estimating the spreading of benzene pollution in rivers under different environmental conditions.

**Source:** German Rapporteur  
**Test condition:** No data  
**Test substance:** No data  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

16-MAR-2004

(868)

**Type:** volatility  
**Media:** water - air  
**Method:** other  
**Year:** 1983

**Method:** Benzene mixed with other volatile organic compounds was applied to a tank containing sea water from the US American Narragansett Bay (with associated planktonic and microbial communities, without sediment). Mixing was carried through four times a day to simulate tidal currents. Different seasons were simulated: spring, summer, winter.

**Result:** Evaporation was found to be the primary loss mechanism for benzene in the winter, with the half-life being 13 days. In spring and summer, the half-lives were 23 and 3.1 days respectively.

**Source:** BP Chemicals Ltd LONDON;  
Deutsche Shell Chemie GmbH Eschborn;  
German Rapporteur

**Test condition:** The half-life for the evaporation of benzene from seawater was investigated in a mesocosm experiment which simulated Narragansett Bay, Rhode Island and contained the associated planktonic and microbial communities.  
Benzene concentrations: 1.4-3.0 ug/l; tank volume: 13 m3;  
spring conditions: temperature 8-16 degree C, summer conditions: temperature 20-22 degree C, winter conditions: temperature 3-7 degree C. Test substance concentrations were monitored by closed-loop stripping and HRGC after adsorption onto charcoal.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (827) (1220)

**Type:** other: deposition to the North Sea.  
**Media:** water - air  
**Method:** other: calculation.  
**Year:** 1989

**Result:** Warmenhoven et al. (1989) calculated the total benzene deposition of European countries to the North Sea to be 420 t/a resulting in iso-deposition lines ranging from 32 g/km<sup>2</sup> a (= 88 ng/m<sup>2</sup>/d) to 3.2 g/km<sup>2</sup> a (= 8.8 ng/m<sup>2</sup>/d). These deposition rates are more than a factor of 1 000 lower than the deposition rates near the emitter.

**Source:** German Rapporteur  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (1238)

### 3.3.2 Distribution

**Media:** air - biota  
**Method:** other (measurement)  
**Year:** 1996

**Method:**

1. Vapours of benzene with radiolabeling ( $^{14}\text{C}$ ) were applied to sterile spinach seedlings in a hermetic chamber containing the phyllosphere (i. e. the stem with the leaves). Carbon dioxide was absorbed by aqueous potassium hydroxide solution, the metabolites of benzene were analysed after the end of the exposure autoradiographically (no details on the identification of the metabolites available, only a reference to an earlier paper).
2. Leaves of mature plants (maple, apple, vine) were floated on water with either the side without stomata or with stomata being exposed to the gas phase. After the end of the exposure, the non volatile metabolites were separated and analysed. The thickness of the waxy epicuticular membrane was determined under the microscope. The amount of wax was determined by washing-off with chloroform.

**Result:**

1. The radioactivity in the spinach leaves was 113,000 counts per minute per gram of fresh weight. Of the total radioactivity, 77 % were low-molecular compounds, 18 % high-molecular compounds, and 5 % were carbon dioxide.
2. The uptake of benzene varies strongly between the stomata and the cuticula (in counts per minute per gram of fresh weight):  
Maple: stomata: 64,000 - 66,000; cuticula (wax membrane: 1.1 - 2.4  $\mu\text{m}$ ): 15,500 - 24,000,  
Apple: stomata: 83,000; cuticula (wax membrane: 2.8  $\mu\text{m}$ ): 21,500,  
Vine: stomata: 42,500 - 49,000; cuticula (wax membrane: 1.0 - 1.7  $\mu\text{m}$ ): 17,500 - 19,000.  
Information on benzene remaining unchanged is not reported.

**Source:** German Rapporteur

**Test condition:**

1. Spinach plants were grown under sterile conditions in a nutrient solution. The benzene concentration in the air was 0.2 mmol/l (= 15.6 mg/l or 15.6 g/m<sup>3</sup>), the exposure time was 72 h at 22-26°C.
2. Leaves of mature plants (maple, apple, vine) were floated on water with either the side without stomata or with stomata being exposed to the gas phase so as to investigate the uptake of benzene through the stomata or the cuticula (coated with wax that absorbs lipophilic substances such as benzene) into the leaves. The benzene concentration was 0.1 mmol/l (= 7.8 mg/l or 7.8 g/m<sup>3</sup>, the exposure time 8 h at 22-23°C in the light (intensity and spectral properties of the light not reported).

**Test substance:** Radiolabeled benzene (3 g; 225.7 MBq/g) was diluted with unlabeled benzene to 30 g. After purification, the specific activity of labeled benzene was 1.76 MBq/mmol (= 22.5 MBq/g).

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

16-MAR-2004 (1178)

**Media:** air - biota - sediment(s) - soil - water  
**Method:** other (calculation): model following first Mackay model

**Method:** An environmental modelling exercise was conducted to estimate benzene's distribution in the UK environment. The computer model used by the Building Research Establishment, developed from Mackay's original model (Mackay D., *Envir. Sci. Technol.* 13, 1218-1223, 1979; Mackay D. & Paterson S., *Envir. Sci. Technol.* 15, 1006-1014, 1981) was used. For the model, it was assumed that 38,000 tonnes benzene are released in the UK per year. Other factors required for the model were molecular weight (78.11), water solubility (1780 mg/l at 20 degree C or 22.8 mg/m<sup>3</sup>), vapour pressure (1.01E2 hPa at 20 degree C), octanol-water partition coefficient (2.13, log; 135, non-log) and the rate constants for photo-oxidation in air (0.03/hour, t<sub>1/2</sub> = 24 hr in polluted air and 4.8E-3/hour, t<sub>1/2</sub> = 6 days in ambient air), photo-oxidation in water (3.6E-4/hour, t<sub>1/2</sub> = 80 days) and biodegradation in water (1.8E-3/hour, t<sub>1/2</sub>=16 days).

**Result:** The distribution of benzene in the environment was estimated to be 0.08-0.49 ug/m<sup>3</sup> in air, 0.42-2.64 ng/l in water, 1.17-7.30 ng/kg in soil, 2.80-17.5 ng/kg in sediment, 2.80-17.5 ng/kg in suspended sediment and 2.73-17.1 ng/kg in biota.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (827)

**Media:** air - biota - sediment(s) - soil - water  
**Method:** Calculation according Mackay, Level III

**Method:** The method is described in detail in Mackay, D. et al., *Chemosphere* 14, 335-374, 1985. Ideally mixed compartments and photolysis (water and air) and biodegradation (water) are as important degradation mechanisms are assumed.

**Result:** For the Netherlands, the model calculation gave following results:

	air	water (fresh)	soil	sediment
concentration	2.1 ug/m <sup>3</sup>	58 ng/l	12 ng/kg	130 ng/kg
distribution	99.0%	0.9%	0.1%	< 0.1%
removal by reaction	4.6%	0.2%	n.c.*	n.c.
removal by advection	95.2%	< 0.1%	n.c.	n.c.

\* n.c. = not calculated  
 Transportation through the air seemed to be the most important removal mechanism.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Model parameters: 41000 km<sup>2</sup> total surface (88% soil/12% water), 1000 m air column, 2.5 m water column, 0.03 m sediment column, 0.15 m soil column, 2.3 d residence time in air, 55% sea air with a benzene level of 0.1 ug/m<sup>3</sup> and 45% land air (mainly from south east direction, i.d. from Belgium and Germany) with a benzene level of 1.8 ug/m<sup>3</sup>.

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (1048)

**Media:** other: air, water, soil  
**Method:** Calculation according Mackay, Level I  
**Year:** 1992

**Result:** Air 99.0%  
Soil 0.1%  
Water 0.9%

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (724)

**Media:** water - soil  
**Method:** other (calculation)  
**Year:** 1982

**Remark:** Based on a reported octanol/water partition coefficient (log Kow) of 2.13 (Hansch C. & Leo A.J. Medchem Project Issue No.26. Pomona College, Claremont, California, 1985 cited in HSDB, 1993), a Koc of 98 was estimated using the equations of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p.4-9. McGraw-Hill, New York, 1982. This value indicates that benzene will be expected to exhibit very high to high mobility in soil (Swann R.L. et al. Res. Rev. 85, 17-28, 1983) and may leach to groundwater.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (530) (1083)

### 3.4 Mode of Degradation in Actual Use

-

### 3.5 Biodegradation

**Type:** aerobic  
**Inoculum:** activated sludge, domestic, non-adapted  
**Concentration:** 17.37 mg/l related to Test substance  
**Degradation:** 96.3 % after 28 day(s)  
**Result:** readily biodegradable

**Method:** OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"  
**Year:** 2000  
**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** The average percent biodegradability of benzene was determined to be 96.3 % (n=3) over a 28 day testing period (99 %, 103 % and 87 % in the single replicates). The 10% biodegradation level was reached between days 3 and 4, and by day 5, biodegradation was 65.13% (n=3). At the end of the 10-day window a degradation of 93 %, 104 % and 86 % was measured. Blank controls showed cumulative background oxygen uptake of 16.87 mg/L over the 28 day test period, which satisfies the OECD guidelines that background oxygen consumption in the blanks not exceed 60 mg/L. Benzene was not inhibitory to the test systems based on the results of the toxicity control experiments.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Test condition:** The aqueous aerobic biodegradability potential of benzene was determined using the OECD (301F) manometric respirometry test method. Biodegradability was determined by measuring the oxygen consumption in a test medium with trace nutrients and inoculated with activated sludge supernatant (unacclimated). Benzene was evaluated at a mean concentration of 17.37 mg/L (carried out in triplicate test systems). The study was conducted at 22 °C ± 1 °C. The positive control substance, sodium benzoate, was evaluated in duplicates at a concentration of 30.29 mg/L. Duplicate toxicity control test systems containing benzene and sodium benzoate at the same concentrations as used alone.

**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
02-SEP-2003 (342)

**Type:** aerobic  
**Inoculum:** other

**Year:** 1998  
**GLP:** no

**Remark:** Aerobic biodegradation of gasoline and its constituents, benzene, toluene and ethylbenzene were studied using an enrichment from soil indigenous microbial population. Gasoline-contaminated soil (obtained from a site in Montreal, Canada) was used to develop an enriched culture over 28 weeks by successive transfer of 10% (v/v) inoculum, amended at each transfer w/ 75 mg/L gasoline (obtained from commercial gas station).

Growth kinetics and gasoline biodegradation were evaluated in 1L culture flasks containing enriched inoculum (10% v/v) and gasoline (16.1 - 660 mg/L) in 120 mL medium during a 4 day period. Liquid or gas samples were removed and analyzed by GC/FID for gasoline component concentrations and potential metabolites (GC/MS). Microbial growth was based on biomass dry weight and absorbance (spectrophotometry at 550 nm) of liquid samples.

Mineralization of <sup>14</sup>C benzene and toluene (initial concentrations 50-200 mg/L) using the enriched culture was investigated individually by analyzing KOH solutions used to trap <sup>14</sup>CO<sub>2</sub> produced from closed test systems. Maximum duration approx 35 days. Test systems containing >100 ppm benzene or toluene were amended 2x/week w/ 2 mLs of oxygen to avoid oxygen deficiency.

Results: The enrichment culture completely degraded 16.1-660 mg/l gasoline in 2.5-16 days respectively, without accumulation of any by-products. Toluene and ethylbenzene showed no lag period in biodegradation, whereas benzene showed a delay in degradation and only began degradation at an increased rate after toluene and ethylbenzene were nearly exhausted. When benzene and toluene were used as sole substrates, the maximum specific rates of their biodegradation were 62.9 and 16.4 times greater than the corresponding values for a mixture (gasoline). The microbial culture was able to mineralize up to 200 mg/l pure toluene and benzene. Maximum mineralization efficiencies of pure benzene and toluene were 76.7 +/- 5.1% and 76.8 +/- 1.3% respectively. Self-inhibition of pure benzene and toluene biodegradation at concentrations above 14.4 and 24.8 mg/L (respectively) is expected to have occurred as biodegradation rates declined at and above these concentrations. Competitive inhibition of specific chemical biodegradation is concluded to have occurred in the gasoline mixture since the maximum specific biodegradation rate decreased when liquid-phase concentrations were above 0.5 mg/L.

**Result:** Maximum specific biodegradation rate (mg/mg biomass/day)

Benzene (in gasoline)	Pure Benzene
0.12	7.55

Maximum liquid-phase concentration (mg/L) supporting highest specific rate of biodegradation

Benzene (in gasoline)	Pure Benzene
0.	

**Source:** Exxon Biomedical Sciences, Inc. East Millstone, N.J. USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Reliability:** (2) valid with restrictions  
29-JUL-2002

(1297)

**Type:** aerobic

**Inoculum:** activated sludge, domestic, non-adapted

**Concentration:** 17 mg/l related to Test substance

**Degradation:** 96 % after 28 day(s)

**Result:** readily biodegradable

**Method:** OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"

**Year:** 2000

**GLP:** yes

**Test substance:** other TS: benzene, purity > 99.9 %

**Result:** This study assessed the "ready" biodegradability of benzene, when tested in triplicate at a concentration of 17 mg/L (51 mg ThOD/L), according to OECD Test Guideline 301F: Manometric Respirometry. After a lag period of 3-4 days, there was rapid and extensive biodegradation of the test material. Biodegradation in the test flasks was a mean of 96% ThOD by the end of the test (Day 28) (106, 82, 101 % for the single replicates), and 88% ThOD by the end of the "10 day window" (93, 81, 90 % for the single replicates). Based on these data, benzene is "readily" biodegradable.

The positive control substance, sodium benzoate, also met the OECD validity criteria of > 60% ThOD by Day 14 (duplicates showed 63, 68% ThOD by Day 3). Blank controls showed cumulative background oxygen uptake of 4.3 mg/L (n=3) over the 28 day test period, which satisfies the OECD guidelines that oxygen uptake in the blanks not exceed 60 mg/L in 28 days. Benzene was not inhibitory to the test systems based on the results of the toxicity control experiments (duplicates showed 86, 91% ThOD by Day 14). The difference of the extremes of replicate values for the test materials at the end of the "10-day window" was 12%, which met the criteria limit of less than 20% difference. This study met all the validity criteria for the OECD 301F "ready" biodegradability test.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Test condition:** The study was conducted at 20.8 °C ± 0 °C. The positive control substance, sodium benzoate, was evaluated in

duplicates at a concentration of 30 mg/L. Duplicate toxicity control test systems containing benzene (17 mg/L) and sodium benzoate (30 mg/L) were also evaluated in the test.

**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
26-JUN-2003 (1031)

**Type:** anaerobic  
**Contact time:** 13 day(s)

**Year:** 1998

**Remark:** Sediment core samples obtained from petroleum contaminated site in Ponca City, CA, were used to evaluate anaerobic biodegradation of benzene by methanogenesis. Benzene is the principal aromatic contaminant, site conc. 130 - 640 uM (10 - 50 mg/L). Sediment samples (30g) were prepared under anaerobic conditions in serum bottles and spiked w/ 0.3-0.5 uCi <sup>14</sup>C benzene (400 - 600 ppb).

**Result:** After 13 days (1st sampling point) 53% of the benzene had been mineralized, degradation indicated by monitoring <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>CH<sub>4</sub> production using gas-proportional counting. <sup>14</sup>CO<sub>2</sub> and <sup>14</sup>CH<sub>4</sub> production were equivalent (%). Extracellular intermediates produced were phenol, acetate, and propionate (LC fractionation/identification).

Rapid conversion of benzene to methane and carbon dioxide occurred in methanogenic sediments. Results are dissimilar to those reported in the literature, where benzene loss in methanogenic sediments occurred after lag periods of 140 to 400 days. Explanations proposed for the extensive and rapid conversion of benzene in this study: 1) prolonged (approx. 50 yrs) microbial exposure to benzene, and 2) tested sediments virtually intact in this study, whereas previous work have diluted sediments w/ excessive aqueous phase (groundwater, media, etc) and reduced competent methanogenic community.

**Source:** Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Reliability:** (2) valid with restrictions  
02-SEP-2003 (1243)

**Type:** aerobic  
**Inoculum:** other: sludge samplings from different sewage plants, rivers, bays and a lake, non adapted  
**Concentration:** 100 mg/l related to Test substance  
**Result:** other: 39 - 41 % (on the upward trend) after 14 days  
**Method:** OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Method:** Inoculum added: 30 mg/l; aniline as reference substance; BOD measurement.

**Remark:** Test procedure according to OECD-Guideline.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (1) valid without restriction

**Flag:** Risk Assessment

02-SEP-2003

(212)

**Type:** aerobic  
**Inoculum:** domestic sewage, non-adapted  
**Concentration:** 2 mg/l related to Test substance  
**Contact time:** 28 day(s)

**Method:** OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"

**Year:** 1981

**Remark:** Within a research and development project the biodegradation of benzene was determined by six different laboratories with the Closed Bottle test (OECD 301 D).

**Result:** The test performed in six different laboratories gave the following results:

1: 51 %, 2: 5 and 56 %, 3: 10 %, 4: 70 %, 5: 88 %, 6: 4 %.

**Source:** German Rapporteur

**Test condition:** Static: dilution water saturated with oxygen is inoculated with the runoff from a wastewater treatment plant. The test substance is added. The bottles are incubated for 5, 15 or 28 days. The oxygen concentration is determined at the beginning and the end of the test. The decrease in oxygen concentration is corrected by the oxygen consumption in a test without test substance and is compared with the theoretical value calculated from the molecular formula.

**Reliability:** (2) valid with restrictions

guideline study with acceptable restrictions

**Flag:** Risk Assessment

02-SEP-2003

(452)

**Type:** aerobic  
**Inoculum:** activated sludge, domestic, non-adapted  
**Concentration:** 17 mg/l related to Test substance  
**Degradation:** 53 - 100 % after 28 day(s)  
**Result:** other: not valid because of over 20 % difference between replicates of the test.

**Method:** OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"  
**Year:** 1999  
**GLP:** yes  
**Test substance:** no data

**Method:** The test was run with three parallel vessels.  
**Result:** Benzene in the tested concentration was inhibitory over the first week of incubation, with oxygen uptake in the test flasks being lower than in the blanks. The lag periods in the three test flasks ranged from 4 to 7 days. At the end of the 10-day window a degradation of 58 %, 72 % and 87 % was measured. After 28 days biodegradation of benzene (measured as ThOD) was 53 %, 80 % and 102 % in the three parallel vessels. As the biodegradation of the replicates differ by more than 20 % the test is regarded as not valid.

**Source:** German Rapporteur  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
16-MAR-2004 (1032)

**Type:** aerobic  
**Inoculum:** activated sludge, domestic, non-adapted  
**Concentration:** 17 mg/l related to Test substance  
**Degradation:** 63 % after 28 day(s)  
**Result:** other: not valid because of over 20 % difference between replicates of the test.

**Method:** OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"  
**Year:** 2001  
**GLP:** yes  
**Test substance:** other TS: benzene, purity > 99 %

**Result:** The average percent biodegradability of benzene was determined to be 63 % (n=3) over a 28 day testing period (54%, 72 % and 63 % in the single replicates). Benzene was not inhibitory to the test systems based on the results of the toxicity control experiments. As the biodegradation of the replicates differ by more than 20 % the test is regarded as not valid.

**Test condition:** The aqueous aerobic biodegradability potential of benzene was determined using the OECD (301F) manometric respirometry test method. Biodegradability was determined by measuring the oxygen consumption in a test medium with trace nutrients and inoculated with activated sludge supernatant (unacclimated). Benzene was evaluated at a concentration of 17 mg/L (carried out in triplicate test

systems). The study was conducted at 22 °C ± 1 °C. The positive control substance, sodium benzoate, was evaluated in duplicates at a concentration of 30 mg/L. Duplicate toxicity control test systems containing benzene and sodium benzoate at the same concentrations as used alone.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
02-SEP-2003 (50)

**Type:** aerobic  
**Inoculum:** activated sludge, industrial  
**Contact time:** 6 day(s)  
**Degradation:** 90 % after 6 day(s)  
**Result:** inherently biodegradable  
**Kinetic:** 2 day(s) 5 %  
6 day(s) 90 %

**Method:** OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"  
**Year:** 1990  
**GLP:** no data  
**Test substance:** no data

**Method:** Static test: liquid phase together with air in a closed bottle.  
**Result:** After the lag phase of 2 days, the degradation was 5 %. In the following 4 days, 85 % were degraded. In all, the degradation was 90 % after 6 days.  
**Source:** German Rapporteur  
**Test condition:** The test substance is dissolved in an aqueous mineral salt medium containing activated sludge from an industrial wastewater treatment plant (dry substance concentration: 1.1 +/- 0.1 g/l). The liquid is aerated by stirring in the bottle. Test substance concentration not given (only a range of 50 - 400 mg DOC/l).

**Reliability:** (2) valid with restrictions  
guideline study without detailed documentation  
**Flag:** Risk Assessment  
26-JUN-2003 (1244)

**Type:** aerobic  
**Inoculum:** activated sludge  
**Concentration:** 50 µg/l related to Test substance  
**Degradation:** = 29 % after 5 day(s)

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Result:** Method: static test in closed system with 14C-labelled test compound; 14C-analysis; microbial inoculum based on activated sludge from microbial sewage treatment; no information about possible pre-acclimation or adaptation.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 25 degree C.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

16-MAR-2004

(634)

**Type:** aerobic  
**Inoculum:** activated sludge, adapted  
**Concentration:** 153 mg/l related to Test substance  
**Degradation:** = 99.9 %

**Method:** other  
**Year:** 1993  
**GLP:** no data  
**Test substance:** no data

**Method:** Complete-mix, bench-scale, continuous-flow activated sludge reactors fitted with stainless steel covers to facilitate off-gas analysis. Retention time was 8 hours. to treat synthetic wastewater with added benzene. Benzene was added to a synthetic waste water containing ethylene glycol, ethyl alcohol, glucose, glutamic acid, acetic acid, phenol, ammonium sulphate, phosphoric acid and salts. The biological activated sludge for initial seeding was obtained from a municipal activated sludge treatment plant, and acclimated to the benzene-containing synthetic wastewater. The activated sludge system was operated at mean cell residence times of 2, 4 and 6 days. Following acclimation, samples were collected over a 60-day testing period and monitored for BOD5, total organic carbon (TOC), COD and benzene (GC analysis). A non-biological control was used to assess stripping.

**Remark:** Test compound not sole source of carbon.

**Result:** The benzene concentration was reduced by 99.9 % (influent concentration: 153 mg/l). 15 % of benzene was found to be stripped from the test system. BOD5, COD and TOC of the synthetic waste water were also measured over a period of 60 days. With a sludge age of 6 days the BOD5 was reduced by 99 %, COD and TOC by 94 %.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions

No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.

**Flag:** Risk Assessment  
02-SEP-2003 (1094)

**Type:** aerobic  
**Inoculum:** activated sludge, domestic, adapted  
**Concentration:** 1000 mg/l related to Test substance  
**Degradation:** = 3.3 % after 6 hour(s)

**Method:** other  
**Year:** 1955

**Remark:** Degradation result given as % BOD of ThOD; respirometric test in Warburg apparatus according to APHA (1946)

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Bench-scale activated sludge (fill and draw operation) was used as the inoculum in the Warburg respirometer at 20°C. At least 14 days acclimation was used together with 50-200 mg feed/l.

**Reliability:** (2) valid with restrictions  
Standard test procedure; no description of method or degradation kinetics.  
02-SEP-2003 (127)

**Type:** aerobic  
**Inoculum:** Arthrobacter sp. (Bacteria)  
**Concentration:** 20 mg/l related to Test substance  
**Degradation:** < .02 % after 7 day(s)  
**Result:** other

**Method:** other  
**Year:** 1974  
**GLP:** no data

**Method:** Benzene utilizing Arthrobacter sp. (B5; B7) were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on glucose. After removing remaining substrate and suspending in mineral salts medium <sup>14</sup>C-labelled benzene was added and the degradation determined by measuring <sup>14</sup>CO<sub>2</sub> release.

**Result:** <sup>14</sup>CO<sub>2</sub> release after 7 d incubation was < 0.02% for both strains.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
No standard test procedure, but in accordance with generally  
26-JUN-2003 (466)

**Type:** aerobic  
**Inoculum:** Nocardia sp. (Bacteria)  
**Concentration:** 20 mg/l related to Test substance  
**Degradation:** = 85 % after 7 day(s)

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Method:** Benzene utilizing Nocardia sp. BR1 were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on the test compound. After removing remaining substrate and suspending in mineral salts medium <sup>14</sup>C-labelled benzene was added and the degradation determined by measuring <sup>14</sup>CO<sub>2</sub> release.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Mineral salts medium.

**Reliability:** (2) valid with restrictions  
No standard test procedure, but in accordance with generally  
26-JUN-2003 (466)

**Type:** aerobic  
**Inoculum:** Pseudomonas sp. (Bacteria)  
**Concentration:** 20 mg/l related to Test substance  
**Degradation:** = 45 - 90 % after 7 day(s)  
**Result:** other

**Method:** other  
**Year:** 1974  
**GLP:** no data

**Method:** Benzene utilizing Pseudomonas sp. (B6/8; Bw9; B4/3) were isolated from soil samples of different origin (field, grassland and forest soils) and precultured on the test compound or on glucose. After removing remaining substrate and suspending in mineral salts medium <sup>14</sup>C-labelled benzene was added and the degradation determined by measuring <sup>14</sup>CO<sub>2</sub> release.  
Nocardia BR1 and Pseudomonas species (B6/8, BW9 and B/3) were cultured in benzene while Arthrobacter species (B5 and B7) and Pseudomonas putida were cultured in p-hydroxybenzoic acid.

**Result:** <sup>14</sup>CO<sub>2</sub> release after 7 d incubation:  
Pseudomonas B6/8 (precultured on benzene) 69%  
Pseudomonas B6/8 (precultured on glucose) 68%  
Pseudomonas Bw9 (precultured on benzene) 90%  
Pseudomonas B4/3 (precultured on benzene) 45%

Further studies were conducted using 100 g of a base-rich, para-brownish, loess (clay) soil from Flachst ckheim near Braunschweig instead of the pure cultures. The soil (pH 7.1, 1.26% carbon and 0.12% nitrogen) was mixed with 2 mg <sup>14</sup>C-labelled benzene dissolved in 0.1 ml acetone, and moistened to 70% of the water capacity. <sup>14</sup>CO<sub>2</sub> production was again measured, after 3 days and 1, 2, 5 and 10 weeks. The

levels of carbon dioxide released were found to be 7.5, 24, 37, 44 and 47% after 3 days and 1, 2, 5 and 10 weeks respectively.

**Source:** BP Chemicals Ltd LONDON;  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Mineral salts medium.

**Reliability:** (2) valid with restrictions  
No standard test procedure, but in accordance with generally  
26-JUN-2003 (466)

**Type:** aerobic

**Inoculum:** other: river and sea water

**Concentration:** 20 mg/l related to Test substance  
10 mg/l related to Test substance

**Method:** other

**Year:** 1987

**GLP:** no data

**Test substance:** no data

**Method:** Cultivation of test substance with river or sea water for 3 days.

**Result:** Benzene in a concentration of 20 mg/l was degraded by 100 % in river water and by 11 % in sea water.  
In a concentration of 10 mg/l, the degradation was 15-30 % in both river and sea water.  
Strongly differing results for River water between the two testing institutes.

**Source:** German Rapporteur

**Test condition:** The following inocula were used: The tests with an initial concentration of 20 mg/l were performed at Showa Pharmaceutical College with water from Tama river (river water) and Enoshima Beach (sea water), the tests with an initial concentration of 10 mg/l at Osaka University with water from Mino river (river water) and Akashi Beach (sea water).

**Reliability:** (4) not assignable  
Japanese (English abstract)

**Flag:** Risk Assessment  
02-SEP-2003 (630)

**Type:** aerobic  
**Inoculum:** other  
**Concentration:** 3 mg/l related to Test substance  
**Degradation:** = 29 % after 20 day(s)  
**Result:** other

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Result:** 24, 27, 24 and 29% bio-oxidation was recorded after 5, 10, 15 and 20 days respectively indicating that benzene is biodegradable.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 13th Edition. American Public Health Association, New York, 1971 was followed. Wastewater from domestic sewage was used as inoculum. Settled domestic wastewater was filtered through glass wool and added as seed material. Aerated dilution water (freshwater) containing minerals and buffer was added to the BOD bottles followed by small aliquots of benzene, at concentrations of 3, 7 and 10 mg/l, taken from 0.1% stock solutions. At least two of the concentrations were tested in duplicate. Dissolved oxygen was monitored periodically. Samples were analysed routinely for ammonia, nitrogen and organic nitrogen which may result in an oxygen demand.

**Reliability:** (2) valid with restrictions  
guideline study without detailed documentation

**Flag:** Risk Assessment  
02-SEP-2003 (910)

**Type:** aerobic  
**Inoculum:** other  
**Concentration:** 480 µg/l related to Test substance  
**Degradation:** = 54.6 % after 48 hour(s)  
**Result:** other

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Method:** Removal of benzene in gasoline-contaminated groundwater collected from a monitoring well in Los Angeles by the autochthonic, petroleum degrading microflora was studied. Influence of additional nitrogen and from contaminated soil enriched microbial inocula to the test culture was investigated. Elimination determined, amount of stripped or adsorbed test substance not measured.

**Remark:** Longer incubation than 20 hours did not result in further elimination, probably due to 35 ug/l benzene being an insufficient carbon source to sustain the microbial population.

**Result:** (I) and (II): <50% benzene removal occurs slowly in

48 hours.  
(III) and (IV): Rapid removal (dropping to 35 µg/l equivalent to 92.7% degradation) of benzene within 20 hours of incubation; longer incubation did not result in further elimination, probably due to 35 µg/l benzene being an insufficient carbon source to sustain the microbial population.  
In samples amended with an inoculum of microflora and with or without nitrogen, degradation was again seen to be rapid, the rates of benzene removal ranging from 5.7 µg/l/hour in the absence of nitrogen to 25.4 µg/l/hour in its presence.

**Source:** Deutsche Shell Chemie GmbH Eschborn,  
German Rapporteur

**Test condition:** Closed batch flask culture with groundwater containing 6.2 mg/l hydrocarbons (a.o. 477 ug/l benzene, 561 ug/l toluene and 153 ug/l xylenes) were incubated with the autochthonic microflora (710 CFU/ml bacteria; 3430 CFU/ml total heterotrophic population):  
(I) sterile control (II) unamended, (III) amended with 100 mg/l NH<sub>4</sub>NO<sub>3</sub>-N and (IV) amended with nitrogen and 1 ml inoculum of a soil enrichment culture with bacteria capable of utilising gasoline as sole carbon source; removal of test substance was followed by GC-analysis.  
Water characteristic: pH 7.93; TOC 23 mg/l; NH<sub>4</sub>-N 0.22 mg/l; NO<sub>3</sub>-N 0.21 mg/l.  
Incubation at approx. 23 degree C, shaken.

**Reliability:** (2) valid with restrictions  
No standard test procedure, but in accordance with generally  
26-JUN-2003 (603)

**Type:** aerobic  
**Inoculum:** other  
**Degradation:** = 80 % after 20 day(s)

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Result:** 58, 67, 76 and 80% bio-oxidation was recorded after 5, 10, 15 and 20 days respectively.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** A mixture of two biologically treated petrochemical effluents, settled domestic wastewater, Kanawha River water taken from the Winfield Locks at Winfield, West Virginia and soil was acclimated to benzene for 45-60 days, and the supernatant used as seed material. Freshwater was used as the dilution water. It is unclear from the paper if the method, conditions and benzene concentrations used in the unacclimated test were used in this case.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-SEP-2003 (910)

**Type:** aerobic  
**Inoculum:** other  
**Concentration:** 1 mg/l related to Test substance  
**Degradation:** > 99 % after 16 day(s)

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** no data

**Method:** Removal of benzene in groundwater samples from Gainesville, Florida (drawn from a 9 m deep shallow). Batch culture experiments according to Bouwer et al., Environ. Sci. Technol. 15, 596-599. Elimination of test substance monitored by GC-MS. Sterile control.

**Result:** After an acclimatization phase of 8 days and a period of slow degradation of 4 days a complete primary degradation of benzene was achieved between day 12 and day 16. No change in benzene concentration in sterile control indicates biological transformation of TS.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Incubation at 20 degree C in the dark; pH 5.3

**Reliability:** (2) valid with restrictions

**Flag:** No standard test procedure, but in accordance with generally Risk Assessment

02-SEP-2003

(292)

**Type:** aerobic  
**Inoculum:** other

**Method:** other  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Method:** The static-culture flask-screening procedure of Bunch R.L. & Chambers C.W. J. Wat. Pollut. Control Fed. 39, 181 (1967) modified by Tabak H.H. & Hannah S. Static Procedure for Biodegradability Determination. Internal EPA Report, Mun. envir. Res. Lab., Cincinnati, Ohio (1979) was followed. Wastewater from domestic sewage was used as inoculum. Benzene was prepared in deionized distilled water as a 10% solution to give concentrations of 5 and 10 mg benzene/l. The solutions were incubated with prechilled dilution water containing 5 mg yeast extract/l, and also with settled domestic wastewater inoculum. The flasks were then incubated for 7 days in glass stoppered bottles (to minimize volatilization) and three weekly subcultures were taken (totalling 28 days of incubation). Duplicate samples at the beginning of each incubation period and triplicate samples at the end of each 7-day period were subjected to GC, total organic carbon (TOC) and dissolved organic carbon (DOC) analyses. The minimum sensitivity of the GC procedures used was about 0.1 mg/l.

**Remark:** Modification of standard test procedure; method in accordance with generally accepted standards and described

in sufficient detail.

**Result:** Benzene demonstrated significant biodegradation with rapid acclimation, 49 and 37% being biodegraded when tested at concentrations of 5 and 10 mg/l respectively after 7 days. Test substance was completely biodegraded at both concentrations in the first and the following subcultures (7 days incubation each).

**Source:** Deutsche Shell Chemie GmbH Eschborn;  
BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** 25 degree C; dark

**Reliability:** (2) valid with restrictions  
02-SEP-2003 (1108)

**Type:** aerobic  
**Inoculum:** other  
**Result:** other

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Method:** Biodegradation of test substance (0, 0.8, 1.6 and 3.2 ul/l) was determined in samples from groundwater, Lester River water and lake water (Lake Superior) by BOD technique according to APHA (1980) Standard methods for the examination of water and wastewater, Washington. DO concentrations were determined at 0, 5, 10, 14 or 15, and 20 days of incubation.

**Result:** After 20 days of incubation approx. 42 % of test substance in river water, 10 % in Superior harbor water and 22 % in ground water were degraded (BOD of ThOD); degradation followed first-order rate kinetics. The rate constants were 0.025/day for groundwater and 0.044/day for river water (lake water not calculated); half-lives for the reaction were 28 and 16 days respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Physicochemical water characteristic:

	ground water	Lester river	Superior harbor
pH	8.23	7.76	7.56
hardness (as CaCO <sub>3</sub> )	121.35	58.59	50.10
Ammonia-N	0.07	0.59	1.03
total solids	170.3	93.2	131.7
dissolved solids	156.7	80.7	109.0
suspended solids	<1.2	<1.2	5.6
bacterial concentration (cells/ml)	55	420	310

**Reliability:** Incubation at 21+/-3 degree C  
(2) valid with restrictions  
Standard test procedure; unclear if degradation results were Risk Assessment

**Flag:** 16-MAR-2004 (1196)

**Type:** aerobic  
**Inoculum:** activated sludge, adapted  
**Concentration:** 1000 mg/l related to Test substance  
**Degradation:** 2.4 % after 5 day(s)

**Method:** other: standard dilution B.O.D. technique according to APHA  
**Year:** 1955  
**GLP:** no data  
**Test substance:** no data

**Method:** Standard test procedure; no description of method or degradation kinetics.  
**Result:** BOD5 = 2.4 % of ThOD.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** Inoculum: activated sludge, adapted for 16 d +/- 48 h, 20°C.  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (127)

**Type:** aerobic  
**Inoculum:** activated sludge, domestic, non-adapted  
**Concentration:** 1000 mg/l related to Test substance  
**Degradation:** 1.9 % after 5 day(s)

**Method:** other: standard dilution B.O.D. technique according to APHA  
**Year:** 1955  
**GLP:** no data  
**Test substance:** no data

**Method:** Standard test procedure; no description of method or degradation kinetics.  
**Result:** BOD5 = 1.9% of ThOD.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** Inoculum: domestic sewage, non adapted, 20°C.  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (127)

**Type:** aerobic  
**Inoculum:** other: Surface water from estuarine river  
**Concentration:** related to Test substance  
**Degradation:** 50 % after 6 day(s)  
**Result:** other: ultimate biodegradation

**Remark:** Concentration: 25 mg/ml  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (678)

**Type:** aerobic  
**Inoculum:** other: mixed microbial populations from an oil refinery settling pond  
**Concentration:** related to Test substance  
**Degradation:** 90 % after 10 day(s)  
**Result:** other: primary degradation observed

**Remark:** Concentration: benzene in 23 microliters of Methanol (100 mg/L final concentration)  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (769)

**Type:** aerobic  
**Inoculum:** other: Lake Superior Harbor water amended with nutrients (nitrogen and phosphorus) and/or acclimated microorganisms  
**Concentration:** related to Test substance  
**Year:** 1986  
**Method:** Biodegradation was measured as BOD related to TOD.  
**Result:** In samples that were either supplemented with nutrients or acclimated microbes no biodegradation of benzene occurred whereas in samples that were amended with nutrients and acclimated microorganisms a rate constant of 0.082 d<sup>-1</sup> and a half-life of 8 days could be determined.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** Concentration: 0.8 to 3.2 microliter/liter (equivalent to 0.7 - 2.8 mg/l).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (1196)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** 50 mg/l related to DOC (Dissolved Organic Carbon)  
**Degradation:** = 0 % after 2.8 month  
**Result:** under test conditions no biodegradation observed  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.  
**Result:** For benzene, NGP was -10±8.9% of the theoretical, indicating a lack of degradation.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** The inoculum was primary digesting sludge collected from Reading Sewage Works (Berkshire, England) which receives a mixture of domestic and industrial wastewaters. Diluted sludge (2-3 g dry solids/l of medium) was incubated with

benzene under a headspace of 90% N<sub>2</sub>-10% CO<sub>2</sub> at 35°C. Sterile controls contained autoclaved sludge and sterile test chemical. Incubation was conducted for at least 60 days. Total gas production was measured weekly. Net gas production (NGP) was expressed as percentage of the theoretical gas production calculated from the stoichiometry of test chemical mineralization to methane and carbon dioxide.

**Test substance:** The test substance was laboratory reagent grade.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
02-SEP-2003 (81)

**Type:** anaerobic  
**Inoculum:** other  
**Degradation:** = 0 % after 161 day(s)  
**Result:** under test conditions no biodegradation observed

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Method:** The microcosms were amended with NH<sub>4</sub>-N, PO<sub>4</sub>-P and one or more of the following compounds: benzene, toluene, phenol, catechol, nitrate and/or biocides. Removal of test substance was determined by GC.

**Remark:** No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.

**Result:** Within an incubation time of 63 or 161 days benzene was neither removed in uncontaminated nor in contaminated samples to a significant degree regardless if it was present as sole source substrate or with other metabolizable compounds.

A second study reported the complete removal of benzene after 6 months following the in situ anoxic biological treatment of a hydrocarbon contaminated aquifer under anaerobic conditions (Battermann G. In: Contaminated Soil. TNO International Conference. p.711-722. Edited by J.W. Assink & W.J. Van den Brink. Nijhoff, Dordrecht, The Netherlands, 1986 (cited in this paper). In the uncontaminated microcosm, benzene was not degraded after 56 days but did inhibit the basal rate of denitrification of the microcosm. No significant degradation was seen in the contaminated microcosm after 161 days but the denitrification processes were unaffected.

**Source:** Deutsche Shell Chemie GmbH Eschborn;  
German Rapporteur

**Test condition:** Removal of benzene (initial concentration 7-8 mg/l) in microcosms filled with aquifer core samples (a) from an uncontaminated site and (b) from the saturated zone below a jet fuel contaminated interval under denitrifying conditions. Incubation under nitrogen atmosphere at 12°C.

**Test substance:** Test substance was reagent grade.  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (544)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** 1 mg/l related to Test substance  
**Degradation:** = 0 % after 96 day(s)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Method:** Removal of benzene in groundwater samples from Gainesville, Florida (raw water intake of a water treatment plant). Batch culture experiments according to Bouwer et al., Environ. Sci. Technol. 15, 596-599. Elimination of test substance monitored by GC-MS. Sterile control.

**Remark:** No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.

Removal determined.

**Result:** No removal during incubation; test terminated after 96 days.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Incubation at 20 degree C in the dark; pH 5.3

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

02-SEP-2003

(292)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** 1 µg/l related to Test substance  
**Degradation:** = 5 % after 7 day(s)  
**Result:** other

**Method:** other  
**Year:** 1990  
**GLP:** no data  
**Test substance:** no data

**Method:** The mineralization of 14C-labelled benzene was followed in a methanogenic sediment microcosm originating from a Dutch river; measurement of 14CO2.

**Remark:** No standard test procedure, but in accordance with generally accepted scientific methods and described in sufficient detail.

**Result:** Total removal of test substance after 7 days was 57-63%  
Incubation for 63 days did not result in higher removal or further mineralization. Sorption to stoppers or the sediment or formation of intermediates were thought to account for most of the removal. Tests with an initial benzene concentration of 100 µg/l had similar results.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Incubation at 20 degree C;  
Sediment characteristics: pH 7.5; 5% organic carbon; 0.42% nitrogen; 0.21% phosphorus; 11% CaCO3.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

02-SEP-2003

(1199)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** 1.17 g/l related to Test substance  
**Degradation:** = 34 % after 4 day(s)  
**Result:** other

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Method:** Acclimation to test substance for 9 month (1.17 g/l benzene, dissolved in 1 ml of methanol; cultures re-fed every 2 months).  
Following acclimation, the subcultures were again fed 1.17 g/l benzene (as only organic carbon source) and incubated, as before, for 2 months. Two parallel cultures, sterile chemical controls without inocula and autoclaved biological controls were used. Seven samples were withdrawn during the incubation period and assessed by GC-MS for the test substance and transformation intermediates.

**Remark:** No standard test procedure but detailed description of method and test conditions; test procedure in accordance with generally accepted scientific standards.

**Result:** After 4 days, the benzene concentration was reduced to 66+/-2% of initial concentration and complete disappearance occurred after 34 days. Phenol (1.88 mg/l) was identified as the major aromatic intermediate with cyclohexanone and propanoic acid also being identified.  
39+/-8% of test substance was dissolved into rubber stoppers.

**Source:** Deutsche Shell Chemie GmbH Eschborn,  
German Rapporteur

**Test condition:** Removal of benzene by a mixed methanogenic inoculum from a stable methanogenic consortia enriched from sewage sludge that degrade ferulic acid to carbon dioxide and methane and had been grown on ferulic acid (as sole carbon and energy source) for 5 years.  
Incubation in a 30% CO<sub>2</sub>-70% N<sub>2</sub> gas atmosphere at 35 degree C in the dark; mineral salts medium with reducing agents, ammonium phosphate as nitrogen source and vitamins.

**Test substance:** Purity: 99.3%.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

02-SEP-2003 (435)

**Type:** anaerobic  
**Inoculum:** other  
**Degradation:** = 72 % after 10 month  
**Result:** other

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** After 20 weeks of incubation at 17°C in the dark, benzene did not show signs of significant degradation. After 40 weeks, the benzene levels were reduced to 28% (i.e. 72% removal) of the originals and after 120 weeks <1% remained (i.e. >99% removal). No evidence of degradation was seen in the autoclaved controls after 40 weeks.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test condition:** Benzene was studied in aquifer material from Oklahoma, USA. The living microcosms tested consisted of anaerobic subsurface materials from aquifers known to support methanogenesis. Autoclaved samples were used as controls.

**Reliability:** (4) not assignable  
02-SEP-2003 (827) (1258)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** .27 mg/l related to Test substance  
**Degradation:** = 72 % after 10 month  
**Result:** other

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Removal of 0.61 mg/l benzene mixed with 0.55 mg/l toluene, 0.27 mg/l ethylbenzene and 0.26 mg/l o-xylene in methanogenic aquifer material. Aquifer samples were collected below a contaminated landfill, which is located on the bank of a river in Oklahoma, USA. GC-analysis after 0, 6, 12, 20, 40 and 120 weeks of incubation. Autoclaved samples were used as controls.  
Results: After 20 weeks incubation benzene did not show signs of significant removal. After 40 weeks, elimination of test substance was 72% and after 120 weeks >99%. No evidence of elimination was seen in the autoclaved controls after 40 weeks.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Incubation at 17 degree C in the dark.  
Characteristic of aquifer water: pH 7.3; 0.7 mg/l nitrate; 218 mg/l ammonia; 344 mg/l TOC.

**Test substance:** High purity  
**Reliability:** (2) valid with restrictions  
02-SEP-2003 (1259)

**Type:** anaerobic  
**Inoculum:** other  
**Concentration:** .12 g/l related to Test substance  
**Result:** other

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Method:** Acclimation to test substance for 9 month (1.17 g/l benzene, dissolved in 1 ml of methanol; cultures re-fed every 2 months). Following acclimation, the subcultures were fed mixtures of unlabelled and <sup>14</sup>C-labelled benzene in separate test sets at concentrations of 0.12 and 0.23 g/l (as sole source of carbon). Individual series of 3 cultures for each concentration, sterile chemical controls without inoculum and sterile controls with autoclaved inoculum were used. During incubation gas (60 days at 25°C in the dark) production was measured, gas composition determined and <sup>14</sup>C activity in both the liquid and headspace assessed.

**Remark:** No standard test procedure but detailed description of method and test conditions; test procedure in accordance with generally accepted scientific standards.  
Used for Risk Assessment.

**Result:** Results:

Benzene concentration	liquid and gas phase	[% mean <sup>14</sup> C activity +/- SD]	
		initial CO <sub>2</sub>	final CO <sub>2</sub>
0.12 g/l			
culture	68+/-1.2	0.002+/-0.0002	5.8 +/-0.36
control	68+/-2.3	0.25 +/-0.25	0.1 +/-0.1
0.23 g/l			
culture	64+/-3.0	0.85 +/-0.044	5.4 +/-0.28
control	57+/-5.2	0.39 +/-0.22	0.17+/-0.17

At the end of the study, 68 and 64% of the <sup>14</sup>C activity was found in the liquid and gas phases and 35 and 31% in the rubber stoppers at 1.5 and 3 mM benzene respectively.

For the tests conducted in the presence of methanol, the corresponding <sup>14</sup>C activity levels in the 1.5 and 3 mM benzene cultures were 53 and 56% in the liquid and gas phases, 41 and 46% in the rubber stoppers, 0.25 and 0.05% in the initial carbon dioxide and 2.6 and 2.0% in the final carbon dioxide respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn;  
German Rapporteur

**Test condition:** Removal of benzene by a mixed methanogenic inoculum from a stable methanogenic consortia enriched from sewage sludge that had been grown on ferulic acid (as sole carbon and energy source) for 5 years.  
Incubation in a 30% CO<sub>2</sub>-70% N<sub>2</sub> gas atmosphere at 35 degree C in the dark; mineral salts medium with reducing agents, ammonium phosphate as nitrogen source and vitamins.

**Test substance:** Purity = 99.3%; ring-labelled test test substance.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
02-SEP-2003

(436)

### **3.6 BOD5, COD or BOD5/COD Ratio**

#### **B O D 5**

**Method:** other  
**GLP:** no data  
**BOD5:** = 0 mg/l  
**Method:** other  
**Year:**  
**GLP:** no data

#### **R A T I O B O D 5 / C O D**

**BOD5/COD:** = 0  
**Method:**  
**Result:** The COD was reported to be 0.25 (no units given).  
**Source:** BP Chemicals Ltd LONDON  
German Rapporteur  
**Test condition:** The standard dilution technique was used with normal sewage as the seed material. No further details on the method of determining the BOD or COD values were available in the citing reference.  
**Reliability:** (4) not assignable  
02-SEP-2003 (710) (1213)  
**Method:** other  
**Year:** 1971  
**GLP:** no data

#### **C O D**

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**COD:** = 2150 mg/g substance

#### **R A T I O B O D 5 / C O D**

**BOD5/COD:** = 1.01  
**Method:**  
**Result:** The BOD5 was reported as 2.18 g/g (converts to 2180 mg/g). The BOD of 2180 mg/g corresponds to 71 % of theoretical oxygen demand (3080 mg/l).  
**Source:** BP Chemicals Ltd LONDON  
German Rapporteur  
**Test condition:** The methods of the APHA. Standard Method for the Examination of Water and Wastewater. No.219. American Public Health Association Inc., New York, (BOD Procedure), 1971 and the ASTM. Annual Book of ASTM Standards. No. D 1252-67. American Society for Testing and Materials, Philadelphia, 1974 were followed. The standard dilution method for determining BOD was conducted at 20 ± 1°C. Allyl thiourea (0.5 mg/l) was added to prevent nitrification. Filtered effluent from a biological sanitary waste treatment plant was used as seed.

The benzene/seed mixture (500 ml, seeded with 10 ml of filtered effluent from a biological sanitary treatment plant) was stirred throughout the study. COD was determined by the standard potassium dichromate method.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

02-SEP-2003 (145)

**Method:** other  
**Year:** 1969  
**GLP:** no data

**Method:** other  
**Year:** 1971  
**GLP:** no data

**R A T I O   B O D 5 / C O D**

**BOD5/COD:** = .53

**Method:**  
**Remark:** Methods: BOD5 = AFNOR T90/103 test (January 1969),  
COD = AFNOR T90/101 test (September 1971),  
TOC = Method according to Davis, L.F., Eng. Bull. Purdue Univ., Eng. Ext. Ser. 132, 989-999 (1968) and Gallion, F., Agence Financiere de Bassin Seine-Normandie (1971).

The inoculum was taken from three polluted surface waters. Test procedures (BOD5 and COD) in accordance with national standard methods and described in sufficient detail.

**Result:** The BOD5 was reported to be 10% of ThOD and the COD was reported to be 19% of ThOD; TOC-removal was 40%.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions  
02-SEP-2003 (302)

**3.7 Bioaccumulation**

**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 96 hour(s) at 21 degree C  
**BCF:** ca. 19  
**Elimination:** yes

**Method:** other  
**Year:** 1998

**Remark:** Both BCF and rate data may be questionable and are ranked as "4" for reliability. This ranking is based on the conclusion that fish metabolism may have been negatively affected, since the additive effects of the total chemical mixture (consisting of benzene, toluene, monochlorobenzene, monobromobenzene and 1,3 dichlorobenzene) had a combined value of 1 based on summation of fLC50 (the individual aqueous concentrations expressed as a fraction of the measured 96h-LC50 values for each chemical) and poor organism health was noted for the duration of the exposure. Several omissions of reported information are noted as follows:

Exposure duration was not reported , although it may be inferred that this was 96 hr.

The ratio of air/water used in the test systems is also not reported. It is unclear whether the test system water was supersaturated with oxygen prior to the test commencing, as the size of the fish and the assumed test duration would have exhausted dissolved oxygen under normal saturated conditions (8 - 9 ppm)

Although the investigators noted poor organism health (sluggishness, erratic swimming) as signs of intoxication, the authors' opinion is that the determined uptake and elimination rates are reasonably accurate estimates which were not significantly influenced by stress due to combined toxic aqueous concentrations. The development of an exposure system suitable for studying the uptake and elimination kinetics in fish of volatile chemicals is discussed. Uptake ( $k_1$ ) and elimination rate ( $k_2$ ) constants in fathead minnows (*Pimephales promelas*) were determined for a mixture of benzene and toluene, and used to estimate bioconcentration factor (BCF<sub>kin</sub>) values.

Exposure experiments with 10 females per exposure were conducted in triplicate. Headspace concentrations of injected benzene & toluene in a closed system (12 L glass dessicator w/ 9L appropriate diluent water) were allowed to achieve steady state between air-water phases prior to addition of fish. Automated sampling and analysis of the air by inline GC/FID provided a concentration-time profile that was then fit to differential equations using numerical integration methods.

Assumptions for the mathematical description of the system are a) instantaneous distribution of chemical between water and air ( $K_{aw}$ ), and b) a first order one-compartment model describing the kinetics of chemical in fish. Rates were derived from the water concentrations, which were determined based on one-compartment analysis (air) with ( $C_w$ ) calculated from GC results of headspace analysis of the system both empty ( $C_{ref}$ ; concentration in air in :M/L) and when containing the water phase ( $C_i$ ).

**Result:**

Uptake rate constants increased with increasing octanol-water partition coefficient ( $K_{ow}$ ), whereas the elimination rate constants were inversely related to  $K_{ow}$ .  $K_{aw}$  values experimentally determined were approximately 26% lower than values reported in the literature. Since steady state concentrations were reached during fish exposure, biotransformation was not expected to have occurred, since chemical concentration did not decrease over time. Uptake rate constants increase

Benzene

$K_{aw}$	$C_w$ mol/L	Log P: Calculated	Log P: Measured
0.224	0.18+/-0.01	48+/-7	2.13

Uptake- $k_1$ , L/kg/hr	Elimination- $k_2$ , (1/hr)	BCF <sub>kin</sub> (ml/g)
7+/-1	0.384+/-0.074	19

The BCF values for benzene and toluene are in general agreement with the range of experimental and calculated data reported in the literature.

**Source:** Exxon Biomedical Sciences, Inc. East Millstone, NJ, USA  
Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (283)

**Species:** Anguilla japonica  
**Exposure period:** 10 day(s) at 20 degree C  
**Concentration:** 50 mg/l  
**BCF:** = 3.5  
**Elimination:** no data

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Remark:** No information about test procedure and test conditions; fish exposed to a mixture of hydrocarbons (crude oil). The test was conducted in seawater and the bioconcentration factor measured in the flesh.

**Result:** A concentration factor of 3.5 was observed after rearing eels in oil-seawater after 10 days. Benzene was no longer detectable in eel flesh after 3 days depuration in clean seawater.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Fish (130-180 g) were treated in 130 l seawater with 60.5 g crude oil (containing 50 mg/l benzene). Benzene was measured in oil suspension and in flesh of the eels (pooled samples from 5 eels) by GC-FID. No further details reported.

**Reliability:** (3) invalid  
Documentation insufficient for assessment  
16-MAR-2004 (853)

**Species:** Carassius auratus (Fish, fresh water)  
**Concentration:** 1 mg/l  
**BCF:** = 4.3  
**Elimination:** no data

**Method:** other  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
No information about test procedure or test conditions. The log bioconcentration factor was found to be 0.63.

**Result:** The concentration of benzene in water as monitored by GC ranged from 0.017 to 0.8 mg/l. The log bioconcentration factor was found to be 0.63. No further details reported.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Goldfish were reared in water containing benzene.  
The concentration of benzene in the fish was measured by gas chromatography after 5 minutes incubation at 80°C of the fish tissue and air.

**Reliability:** (3) invalid  
16-MAR-2004 (854)

**Species:** Clupea harengus (Fish, estuary, marine)

**Exposure period:** 48 hour(s)

**Concentration:** 88 µg/l

**Elimination:** no data

**Method:** other

**Year:** 1977

**GLP:** no data

**Test substance:** no data

**Result:** Benzene concentration in the seawater after 48 h was 69 % of the initial concentration. 6 hours after start of exposure and then daily for 7 days gallbladder, intestine, pyloric caeca, gill, brain, liver, muscle, kidney and immature male and female gonad tissues were sampled and analyzed for 14C. The following BCFs were determined: 31 (gallbladder), 7 (gills), 6 (intestine, pyloric caeca, brain), 5 (liver), 4 (muscle, kidney) and 2 (gonad). After 5 days of depuration only in the gallbladder 14C was found. In all other tissues a fast depuration was observed.  
A second experiment was conducted to find out whether benzene or metabolites was accumulated in the gallbladder. 6 fish were exposed to 100 nl/l benzene. After 48 h the gallbladder was extracted and the extract was analyzed for benzene by GC. No detectable benzene (detection limit: 0.1 nl/g) was found. This indicated that most or all of the 14C measured in the gallbladder was not due to benzene but to one or more metabolites.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
German Rapporteur

**Test condition:** A static exposure of the fish to 100 nl/l benzene for 48 h was preceded and followed by a continuous water flow of 2 l/min. Radiometric analysis of water samples was conducted at 0, 6, 24 and 48 h.  
Salinity: 24 ppt; temperature: 9-11 degree C

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
16-MAR-2004 (633)

**Species:** Clupea harengus (Fish, estuary, marine)  
**Elimination:** no data

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Purity/radiochemical purity of test substance, no. of exposed animals not reported; no statistical analysis; since only <sup>14</sup>C-uptake was measured, no discrimination between benzene and its metabolites possible.  
Non-feeding Pacific herring larvae (no. of animals not reported), obtained from naturally fertilized eggs and incubated in the laboratory, were exposed five days after hatching, when yolk was totally consumed, to <sup>14</sup>C-labelled benzene in static filtered seawater. Actual initial concentrations of benzene in were determined by GC. Benzene concentrations in water decreased to ca. 37% of initial conc. within 24 h; no <sup>14</sup>C was detected after 48 h. No analysis for <sup>14</sup>C-labelled metabolites was performed.

Results (after 6 to 12 h exposure):

Initial conc. of benzene in water [mg/l]	<sup>14</sup> C-label in larvae (calculated as benzene) [mg/l]
0.127	0.202
1.05	2.82
1.85	3.60

After 12 h, <sup>14</sup>C-label gradually leveled off or near initial exposure concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Filtered seawater; salinity 23 g/l; oxygen near saturation level; temperature 12.2-13.5 degree C.

**Reliability:** (3) invalid  
16-MAR-2004

(329)

**Species:** Clupea harengus (Fish, estuary, marine)  
**Elimination:** no data

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Remark:** Purity/radiochemical purity of test substance, no. of exposed animals not reported; no statistical analysis; since only <sup>14</sup>C-uptake was measured, no discrimination between benzene and its metabolites possible.  
Used for Risk Assessment.

**Result:** Eggs and yolk-sac larvae accumulated benzene quickly within 6 to 12 h reaching maximal BCF values of about 11. Also non-feeding postyolk-sac larvae absorbed benzene quickly within 6 to 12 h. The highest BCF of 2.6 was found at an initial benzene concentration of 1.2 µl/l. Postyolk-sac larvae feeding on contaminated rotifers or rotifers in contaminated

water demonstrated a bimodal uptake. Maximum tissue concentrations of 8.16 µl/l were found after 72 h in fed larvae exposed to an initial benzene concentration of 2.1 µl/l. This means a BCF of about 4. Larvae that were exposed only via rotifers previously contaminated with 1.2 µl/l benzene experienced no initial rapid accumulation. Rather a steady rise in contamination to 0.31 µl/l was exhibited.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Eggs and larvae of pacific herring (*Clupea harengus pallasii*) were exposed to 14C-labelled benzene. Eggs and yolk-sac larvae were exposed through the water phase whereas postyolk-sac larvae were both exposed via benzene-treated water and benzene-treated live food. As food source the marine rotifer *Brachionus plicatilis* was used. To contaminate the food rotifers were allowed to accumulate benzene for 48 h. The benzene concentration in the exposed rotifers was not measured. However, previous experiments conducted by the authors found BCF values related to 14C of 103 to 104 for periods up to 8 days for the rotifers. This high accumulation may be due to the fact that the rotifers are not able to discharge or metabolize benzene. Eggs and larvae of pacific herring were exposed for 48 respectively 72 h. After 24 h the benzene concentration in the water decreased to about 50 to 23 % of the initial concentration. After 48 h no benzene was detectable.  
Filtered seawater; salinity 23 g/l; oxygen near saturation level; temperature 12.2-13.5 degree C.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
16-MAR-2004 (328)

**Species:** *Leuciscus idus melanotus* (Fish, fresh water)  
**Exposure period:** 3 day(s)  
**Concentration:** .05 mg/l  
**BCF:** < 10  
**Elimination:** no data

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Method:** The methods of Freitag D. *Ecotoxic. envir. Saf.* 6, 60-81, 1982 and Korte F. et al. *Chemosphere* 7, 79-102, 1977 were followed: 5 fish (2-5 g) were exposed to 14C-labeled benzene in a closed system and concentrations determined by following radioactivity in fish and water; BCF-values related to wet weight.

**Source:** German Rapporteur  
**Test condition:** Temperature 23+/-3 degree C; pH 7; hardness 100 mg CaO/l  
**Test substance:** Purity: >98%.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (375) (636)

**Species:** Morone saxatilis (Fish, estuary, marine)  
**Exposure period:** 2 day(s)  
**Elimination:** no data

**Method:** other  
**Year:** 1987  
**GLP:** no data  
**Test substance:** no data

**Remark:** The half-life (t<sub>1/2</sub>) was found to be <1 day, indicating a moderate tendency for benzene accumulation.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Fish were exposed to benzene in water and the concentration of benzene in the muscle tissue was followed over a 7-day clearance period.

**Reliability:** (4) not assignable  
16-MAR-2004 (827) (830)

**Species:** Morone saxatilis (Fish, estuary, marine)  
**Exposure period:** 48 hour(s)  
**Concentration:** .06 mg/l  
**Elimination:** yes

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Remark:** During static exposure, the temperature was not controlled. The counting efficiencies for 14-C were determined by internal standardisation and were 85 % for water samples and 67 % for fish samples.

**Result:** Accumulation was determined 6 hours, 1 day and 2 days after the start of exposition:  
After 6 hours: 7.16 (brain), 1.11 (muscle), 1.14 (fat), 2.95 (heart), 2.72 (stomach), 9.77 (liver), 5.45 (intestine), 14.8 (colon).  
After 1 day: 5.57 (gill).  
After 2 days: 53.4 (gall bladder).

Depuration after the beginning of flow-through of water (in terms of 14-C activity):  
Below determination limit after 1 day: muscle and stomach.  
Below determination limit after 2 days: heart and intestine.  
Below determination limit after 3 days: colon.  
Below determination limit after 4 days: gill and brain.  
Below determination limit after 5 days: gall bladder.  
Remaining activity after 7 days: 3.0 % (fat) and 14 % (liver).

**Source:** German Rapporteur

**Test condition:** Fish were exposed in seawater to benzene concentrations of 0.06 mg/l for 48 h under static conditions. After exposure fish were held under flow-through conditions in filtered seawater to study depuration. The benzene concentrations in seawater employed in the experiments dropped to 30 - 43 % of their initial values within 48 hours of the start of the

exposure experiments.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
16-MAR-2004 (556) (631)

**Species:** Salmo gairdneri (Fish, estuary, fresh water)  
**BCF:** = 19  
**Elimination:** no data

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** The log bioconcentration factor in rainbow trout muscle was calculated to be 1.28.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Salmo gairdneri/Oncorhynchus mykiss were tested. No further details were available.

**Reliability:** (4) not assignable  
16-MAR-2004 (140) (331)

**Species:** other  
**BCF:** 6 - 24  
**Elimination:** no data

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Based on reported log Kow of 2.13 (Hansch C. & Leo A.J. Medchem Project Issue No. 26. Pomona College, Claremont, California, 1985 cited in HSDB, 1993), a bioconcentration factor of 24 was estimated using the methods of Lyman W.J. et al. Handbook of Chemical Property Estimation Methods. p.5-5. McGraw-Hill, New York, 1982 (cited in HSDB, 1993). Using this same log Kow value and Mackay's empirical relation (Mackay D. Envir. Sci. Technol. 16, 274-278, 1982 cited in BUA, 1988), the bioconcentration factor was estimated to be about 6, signifying a low bioaccumulation potential.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (4) not assignable  
16-MAR-2004 (162) (530) (1084)

**Species:** other: Chlorella fusca (green algae)  
**Exposure period:** 24 hour(s)  
**Concentration:** .05 mg/l  
**BCF:** = 30  
**Elimination:** no data

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Algae (20 mg d.w.) were exposed to 50 µg/l 14C-labelled benzene for 24 hours. After this time algal cells were separated by centrifugation and the radioactivity was measured in the algae and in the supernatant. BCF-values was related to wet weight. Temperature 20-25 degree C; illumination 16 h/day.

**Test substance:** Purity: >98%.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (375) (406) (636)

**Species:** other: Daphnia pulex  
**Exposure period:** 24 hour(s)  
**Elimination:** yes

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Result:** Bioconcentration factors of 225 (uptake from water alone), 203 (uptake from preloaded algae) and 153 (uptake both from water and algae) were found. It is unknown, whether the bioaccumulation factors were related to wet or dry weight. After 72 hours 88 % of the accumulated benzene was lost by the daphnids exposed solely via the water phase and 83 % by the daphnids exposed to both water and algae

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Filtered lake water; pH 7.5; temperature 13-17 degree C. 20 daphnids per set were employed. The uptake of benzene was tested under three conditions: from water alone, from algae (Ankistrodesmus falcatus) preloaded with benzene and from a medium in which both water and algae were contaminated. The concentration of benzene in water was 0.04 µg/l. Ankistrodesmus falcatus was preloaded with benzene by exposing the algae for 48 hours to a benzene concentration of 50 µg/l. After repeated washing the algae were dosed to the daphnids at a density of 104 algal cells per milliliter. The accumulated benzene concentration was 1.5 pg per algal cell. The daphnids were exposed to the different systems for 24 hours. After this time they were placed into a clean medium solution for 72 hours to examine the clearance

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
16-MAR-2004 (1160)

**Species:** other: *Engraulis nordax* (Northern anchovies)  
**Elimination:** yes

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** other TS: 14-C labeled, 99.9 %

**Result:** Accumulation after 6 hours, 1 and 2 days was as follows (tissue examined and test concentrations in mg/l):  
After 6 hours: 7.1 (gill, 0.0006), 7.9 (gill, 0.004), 7.5 (brain, 0.004), 9.1 (brain, 0.0006), 29.9 (muscle, 0.009).  
After 1 day: 41.8 (gill and brain, 0.10), 113 (gill and brain, 0.009), 10 (muscle, 0.10), 22.7 (muscle, 3.3), 45.1 (liver, 3.3).  
After 2 days: 34.3 (gill, 3.3), 30.0 (brain, 3.3), 31.9 (liver, 0.0006), 309 (liver, 0.009), 229 (gall bladder, 0.004), 8450 (gall bladder, 0.009), 34.8 (intestine, 0.0006), 505 (intestine, 0.009).

Depuration after the beginning of flow-through of water (in terms of 14-C activity): Remaining activity after 2 days (in %; test concentrations: 0.10 / 0.009 mg/l):  
Gill: 61 / 26  
Brain: 22 / 42  
Muscle: 93 / not detectable  
Liver: 70 / 13  
Gall bladder: 69 / 63  
Intestine: 70 / 17.

**Source:** German Rapporteur  
**Test condition:** Fish were exposed in seawater to benzene concentrations of 0.6 µg/l to 3.2 mg/l for 48 h under static conditions. After exposure fish were held under flow-through conditions in filtered seawater to study depuration. The benzene concentrations in seawater employed in the experiments dropped to 30 - 43 % of their initial values within 48 hours of the start of the exposure experiments.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
16-MAR-2004 (631)

**Species:** other: Tapes semidecussata (Bivalvia)  
**Exposure period:** 8 day(s)  
**Concentration:** 1.6 mg/l  
**BCF:** = 0  
**Elimination:** no data

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Result:** Benzene concentration in the exposed clams was below the detection limit (0.6 mg/l) during the whole exposure period.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 70 Manila clams (size class ca. 20 ml in volume, collected from the sea) were exposed to the water-soluble fraction of crude oil (total 3.1 mg/l) which contained a mixture of 6 monoaromatics (toluene, ethylbenzene, o-, p-, m-xylene and 0.9-2.2 mg/l benzene). Exposure in a continuous-flow system under natural environmental conditions. The amount of aromatics in water was measured three times a day, tissue content every 48 h in pooled tissues of 10 clams by GC-FID. Filtered sterilized seawater; salinity 30 g/l; temperature 14+/-1 degree C; light intensity, wavelenght and photoperiod approximating natural conditions.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (846)

**Species:** other: activated sludge  
**Exposure period:** 5 day(s) at 25 degree C  
**Concentration:** .05 mg/l  
**BCF:** = 1700  
**Elimination:** no data

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Activated sludge from a municipal sewage treatment plant (1 g dry weight/l) was exposed to 14C-labeled benzene in a closed system and radioactivity measured after incubation; nutrient solution and sludge replenished once a week; BCF related to dry weight.  
Synthetic nutrient solution; constant stirring.

**Test substance:** Purity: >98%.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
16-MAR-2004 (375) (636)

### 3.8 Additional Remarks

**Memo:** Anaerobic biodegradation of BTEX and gasoline in various aquatic sediments.

**Remark:** The extent of biodegradation of benzene, toluene, ethylbenzene and the three isomers of xylene (BTEX) as a mixture and from gasoline was examined in four different sediments: the New York/New Jersey Harbor estuary (polluted); Tuckerton, N.J. (pristine); Onondaga Lake, N.Y. (polluted) and Blue Mountain. Lake, N.Y. (pristine). Enrichment cultures were established with each sediment using denitrifying, sulfidogenic, methanogenic and iron reducing media, as well as site water. BTEX loss, as measured by GC-FID, was extensive in the sediments which had a long history of pollution, with all compounds being utilized within 21-91 days in the most active cultures, and was very slight or non-existent in the pristine sediments. Also, the pattern of loss was different under the various reducing conditions within each sediment and between sediments. For example benzene loss was only observed in sulfidogenic cultures from the NY/NJ Harbor sediments while toluene was degraded under all redox conditions. The loss of BTEX was correlated to the reduction of the various electron acceptors. In cultures amended with gasoline the degradation was much slower and incomplete. These results show that the fate of the different BTEX components in anoxic sediments is dependent on the prevailing redox conditions as well as on the characteristics and pollution history of the sediment.

Additional Comments: Each of the aquatic sediment cultures was amended with BTEX at a concentration of 100 microM (benzene =7.8 mg/L). Benzene degradation was observed only in cultures from one site, Arthur Kill (NY/NJ), and only under sulfidogenic conditions. Benzene was the slowest of the aromatics to be utilized, while toluene was the first compound to be lost in all the cultures followed by ethylbenzene and m-xylene in denitrifying cultures and o- and m-xylene in sulfidogenic cultures (NY/NJ). These findings indicate that the fate of BTEX in anaerobic sediment is dependent on the characteristics of the site, its pollution history and the terminal electron acceptor available.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Reliability:** (2) valid with restrictions  
16-MAR-2004

(890)

**Memo:** Exposure of agricultural soils by sewage sludges.

**Result:** In table I of this paper, concentrations of benzene in sewage sludge are reported. The concentration unit is not reported but is most likely to be µg/kg:  
Range: 0.053 - 11.3; median (50 %): 0.32.

**Source:** German Rapporteur  
**Flag:** Risk Assessment

16-OCT-2002

(1256)

**Memo:** Fate of benzene in the global atmosphere.

**Result:** The model EXATM divides the global atmosphere in different parts differentiating between troposphere and stratosphere as well as polar / temperate zones and (sub-) tropical zone and between northern and southern hemisphere. This division into different parts and the exchange rates between these parts have been investigated by the global distribution of 14-C.

By taking into account a constant input of 645 570 t/a, the equilibrium of concentrations in the tropospheric and stratospheric parts of the atmosphere depends upon the half-life of benzene in the atmosphere: with a half-life of 13 days, the equilibrium is reached after about 115 days, with a half-life of 22 days, the equilibrium is reached after about 190 days, and without any degradation in the atmosphere, a stationary state would be reached only after 5.6 years.

Taking into account the rapid photochemical degradation of benzene, a significant transport into the stratosphere appears to be unlikely.

**Source:** German Rapporteur

**Flag:** Risk Assessment

16-OCT-2002

(90)

**Memo:** Formation of benzene by anoxic dechlorination of chlorinated benzenes.

**Method:** An equimolar mixture of trichlorobenzenes was added to the anaerobic culture and incubated. The metabolisation of the trichlorobenzenes was followed analytically by extraction of centrifuged aliquots from the culture medium and GC-MS analysis.

**Result:** Trichlorobenzenes were dechlorinated to dichlorobenzenes and chlorobenzene.

Adaptation phase:

After the tri- and dichlorobenzenes had disappeared after about 25 days, benzene began to appear in the culture medium as final dechlorination product.

After 50 days, about 5 mol% of the trichlorobenzenes added (0.8 mmol/l) had been converted into benzene (reading from a diagramm).

After this adaptation, the inoculum was able to eliminate trichlorobenzenes concentrations of about 0.5 mmol/l within 20 days completely with stationary benzene concentrations remaining almost constant.

**Source:** German Rapporteur

**Test condition:** Chlorinated benzenes in acetone solution was added to a mixed methanogenic culture enriched from sediment samples from the Saale river near Jena at 28°C in the dark for over 1 year.

**Test substance:** > 99.9 % purity (Promochem, Wesel, Germany)

**Flag:** Risk Assessment

16-OCT-2002

(841)

**Memo:** Global distribution of benzene from Alaska to the South Pole.

**Method:** Grab sampling, kryo-focussing, GC-FID on a 30 m SE-30 fused silica column.

**Result:** Barrow, Alaska / USA, 71 °N: 58 measurements (3/82 - 5/83):  
3 % of values below detection limit (MDL) (5 pptV)  
overall average concentration: 167 pptV  
average of concentrations above MDL: 167 +/- 19 pptV (+/- 90 % confidence limits)  
115 % variation between summer (low values) and winter (high values)

Cape Meares, Oregon / USA, 45 °N: 62 measurements (12/80 - 3/83): no values below detection limit (MDL) (5 pptV)  
overall average concentration: 230 +/- 36 pptV (+/- 90 % confidence limits)  
107 % variation between summer (low values) and winter (high values)

Niwot Ridge, Colorado / USA, 42 °N: 47 measurements (5/82 - 3/83): 2 % of values below detection limit (MDL) (5 pptV)  
overall average concentration: 119 pptV  
average of concentrations above MDL: 122 +/- 26 pptV (+/- 90 % confidence limits)  
104 % variation between summer (low values) and winter (high values)

Whiteface Mountain, New York / USA, 44 °N: 34 measurements (8/82 - 3/83): no values below detection limit (MDL) (5 pptV)  
overall average concentration: 262 +/- 46 pptV (+/- 90 % confidence limits)  
90 % variation between summer (low values) and winter (high values)

Mauna Loa, Hawaii / USA, 20 °N: 31 measurements (4/82 - 4/83):  
19 % of values below detection limit (MDL) (5 pptV)  
overall average concentration: 100 pptV  
average of concentrations above MDL: 124 +/- 43 pptV +/- 90 % confidence limits)  
9 % variation between summer (low values) and winter (high values)

Samoa, Western Pcific, 14 °S: 47 measurements (5/82 - 3/83):  
23 % of values below detection limit (MDL) (5 pptV)  
overall average concentration: 57 pptV  
average of concentrations above MDL: 75 +/- 15 pptV (+/- 90 % confidence limits)  
30 % variation between summer (low values) and winter (high values)

Cape Point, South Africa, 35 °S: 8 measurements (5/82 - 3/83)  
13 % of values below detection limit (MDL) (5 pptV)  
overall average concentration: 53 pptV  
average of concentrations above MDL: 62 +/- 32 pptV (+/- 90

% confidence limits)

Cape Grim, Tasmania / Australia, 42 °S: 14 measurements  
(5/82 - 3/83): 40 % of values below detection limit (MDL) (5 pptV)

overall average concentration: 31 pptV  
average of concentrations above MDL: 55 +/- 36 pptV (+/- 90 % confidence limits)

South Pole, 90 °S: Benzene not detected between 1/80 and 1/83

Conclusions:

1. Benzene concentrations increase from south to north and are highest in the northern hemisphere. The average northern hemisphere concentrations drops off rapidly at 44° N as one moves southward.
2. The benzene concentrations at four North American sites are inversely proportional to the seasonal cycle of OH radical concentrations which are high in summer and low in winter according to solar radiation intensity, i.e. winter time benzene concentrations are highest and summer concentrations are lower by about a factor of 3.
3. The latitudinal profile of benzene is consistent with the expected locations and sources. Rasmussen and Khalil (1983) concluded that the benzene atmospheric lifetime is expected to be around 10 to 20 days during summer at northern high latitudes and about 4 days in tropical regions.

**Source:** German Rapporteur

**Test condition:** Temperature-programmed gas chromatograph

**Test substance:** No data

**Flag:** Risk Assessment

16-MAR-2004

(926)

**Memo:** Photochemical reaction of benzene to phenol, nitrobenzene and ring-opening products.

**Method:** No data (collection of results from other papers)

**Result:** The reaction rate constant of benzene with OH radicals is  $1.2 \times 10^{(-12)}$  cm<sup>3</sup> / molecule / s.

The benzene-OH adduct may react with NO<sub>2</sub> to form nitrobenzene and with molecular oxygen to form phenol with a product ratio of nitrobenzene : phenol = 1 : 2.

Ring opening products account for 20 % of the products (formaldehyde, formic acid, maleic anhydride, glyoxal), phenol and nitrobenzene for 80 % of the products, among them 27 % nitrobenzene and 53 % phenol.

With benzene concentrations of 5 - 30 ppb (typically 10 ppb) and OH radical concentrations of  $10^{(-7)}$  ppm in Los Angeles (published 1981 - 1984) and a reaction rate constant of  $1.8 \times 10^{(-3)}$  / ppm / min, a removal rate for benzene of 0.1 ppb / h by reaction with OH radicals is calculated.

**Source:** German Rapporteur

**Test condition:** No data

**Test substance:** No data

**Flag:** Risk Assessment

16-MAR-2004

(450)

**Memo:** Photochemical reaction products of benzene and nitrogen monoxide.

**Method:** Irradiation by a Xenon lamp of a gaseous mixture of benzene (2000 ppm) and NO (1000 ppm) in the gas phase at 25 - 30 °C for 5 hours, extraction of the solid reaction products with methanol, analysing the extract with thin-layer chromatography (TLC), after methylation with diazomethane with gas chromatography - electron capture detector (GC-ECD) and gas chromatography - mass spectrometry (GC-MS).

**Remark:** Used for Risk Assessment.

**Result:** TLC of the extract together with authentic samples showed the presence of o- and p-nitrophenol as well as of 2,4- and 2,6-dinitrophenol. This was confirmed by GC-MS. The quantification of the products after the reaction of 10 % of the original amount of benzene showed the following results: 15,6 % of the conversion products were converted into nitrobenzene and nitrophenols, which in turn were nitrobenzene (5.5 %), o-nitrophenol (2.9 %), p-nitrophenol (4.8 %), 2,4-dinitrophenol (1.3 %), and 2,6-dinitrophenol (1.1 %).

Temperature and wave lengths employed appear to be relevant for processes in the atmosphere.

**Source:** German Rapporteur

**Test condition:** The test was performed in a 1 l quartz vessel with wave lengths > 300 nm. No further details were given on the gas phase; there is a reference to a previous paper (Nojima K et al., Chemosphere 5: 247 - 252, 1974).

**Test substance:** No data

**Flag:** Risk Assessment

16-OCT-2002

(837)

**Memo:** Ring-fission products of o-cresol as primary oxidation product of toluene in analogy to benzene.

**Method:**

1. Smog chamber experiments: sunlight irradiation on a 4 to 80 cu m chamber covered with Teflon film.
2. Smog-chamber experiments with ozone and nitrogen dioxide in the dark: Reaction in a 4 cu m chamber protected against sun irradiation.
3. Photooxidation experiments were done with pyruvic acid and biacetyl, two products of o-cresol oxidation.

**Result:**

1. In an experiment with an 80 cu m chamber running for 5.5 hours at 23 °C containing initial concentrations of 0.61 ppm o-cresol, 0.161 ppm nitrogen monoxide, and 0.038 ppm nitrogen dioxide, the following was observed: ozone (90 ppb maximum), 56 % of o-cresol had not reacted, and 12 % of nitrogen oxides had not reacted. Particulate matter (maximum: 204 cu µm/cu cm) was also observed.
2. In an experiment with a 4 cu m chamber running for 4 hours at 17 °C containing initial concentrations of 1.07 ppm cresol, 0.28 ppm nitrogen dioxide, and 0.17 ppm ozone, it was found that 46 % of the o-cresol, 0.14 of 0.17 ppm ozone, and 0.242 of 0.28 ppm nitrogen dioxide had reacted.

3. Pyruvic acid and biacetyl reacted only in presence of nitrogen oxides under influence of sunlight to produce peroxyacetylnitrate: in one run, from 0.72 ppm carbonyl (pyruvic acid), in 4 hours, a maximum concentration of 35 ppb of peroxyacetylnitrate was formed.

The methyl group of cresol appears not to be involved in the photochemical oxidation, so the reaction mechanism in terms of substitution with hydroxyl and nitro groups may also be valid for the degradation of benzene.

**Source:** German Rapporteur

**Test condition:** 1. Smog chamber containing ozone-free air were filled with nitrogen oxides and cresol diluted with nitrogen and exposed to sunlight in outdoor experiments for 3 - 5,5 hours.  
2. Ozone, o-cresol, and nitrogen dioxide were injected into the chamber protected against sun light and containing, ozone-free purified air for 2 - 4 hours. Control experiments with cresol in purified air alone or nitrogen oxides and ozone alone in air were also performed.  
3. Pyruvic acid and biacetyl alone in pure air (< 14 ppm nitrogen oxides) in the dark or in sunlight and a mixture of pyruvic acid or biacetyl with nitrogen oxides in sunlight were allowed to react for 2 to 5 hours to produce peroxyacetylnitrate.

**Test substance:** No data

**Flag:** Risk Assessment

16-MAR-2004

(449)

**Memo:** Ring-fission products of o-cresol as primary oxidation product of toluene in analogy to benzene.

**Method:** Smog chamber experiments: sunlight irradiation on a 4 or 60 cu m chamber covered with Teflon film.

**Remark:** Used for Risk Assessment.

**Result:** In an experiment with a 60 cu m chamber running for 5.5 hours containing initial concentrations of 0.36 ppm nitrogen monoxide, 0.30 ppm nitrogen dioxide and 0.70 ppm o-cresol, the following products were observed: 156 ppm ozone (after 5.5 hours), 7 ppb peroxyacetylnitrate, formaldehyde (31 ppb after 2 h, 47 ppb after 4 h), acetaldehyde (17 ppb after 2 h, 15 ppb after 4 h), pyruvic acid (not quantified), and particulate matter (460 µg/m<sup>3</sup>) consisting of hydroxynitrotoluenes, hydroxydinitotoluenes, and dihydroxynitrotoluenes.

The methyl group of cresol appears not to be involved in the photochemical oxidation, so the reaction mechanism in terms of substitution with hydroxyl and nitro groups may also be valid for the degradation of benzene.

**Source:** German Rapporteur

**Test condition:** Smog chamber containing ozone-free air were filled with nitrogen oxides and cresol diluted with nitrogen and exposed to sunlight in outdoor experiments for 3 - 6 hours.

**Test substance:** No data

**Flag:** Risk Assessment

16-OCT-2002

(448)

**Memo:** Transport of benzene by oceanic and continental air masses to Brittany / France.

**Method:** Sampling of non-methane hydrocarbons on Carbotrap, cryo-focussing, GC-FID on a 25 m capillary column. Automated system performing GC-analyses every 2 hours. Back-tracking of individual air masses in terms of their geographic origin and analysing their NMVOC content.

**Result:** Overall monthly average benzene concentrations at Porspoder (Brittany) (pptV): 350 (2/82), 280 (3/82), 230 (4/82), 360 (5/82), 300 (6/82), 120 (7/82), 100 (8/82), 87 (9/82), 71 (10/82), 79 (11/82), 92 (12/82), 96 (1/83), 220 (2/83)

Continental air masses coming from St. Petersburg over the European continent (pptV): 370 (4/82), 580 (5/82), 330 (6/82), 220 (7/82), 180 (10/82), 190 (11/82), 230 (2/83)

North Sea air masses, coming from the North Sea via the English channel (pptV): 500 (4/82), 300 (6/82), 100 (10/82), 470 (1/83)

England air masses, coming from the British isles (pptV): 260 (4/82), 160 (5/82), 400 (6/82), 140 (7-8/82), 28 (9/82), 47 (10/82), 180 (12/82), 150 (2/83)

Stagnant oceanic air masses, staying one day or more near the coast before reaching Porspoder (pptV): 210 (4/82), 240 (5/82), 260 (6/82), 38 (7/82), 130 (8/82), 120 (9/82), 59 (10/82), 74 (11/82), 91 (12/82), 130 (1/83), 52 (2/83)

Oceanic air masses, coming from the Atlantic ocean with fast wind speeds up to 90 km/h (pptV): 110 (4/82), 160 (5/82), 65 (6/82), 8 (8/82), 18 (9-10/82), 59 (11/82), 43 (1/83), 62 (2/83)

These results allow the following conclusions:

There are seasonal variations of benzene concentrations: August 1992 - January 1993: 71 - 100; February - June 1992: 230 - 360 pptV (all directions). The maximum (spring) / minimum (autumn) ratio is about five. This ratio reflects the high OH concentration in summer and the low OH concentration in winter that scavenges benzene in the troposphere.

The concentrations in air masses from continental Europe are higher by a factor of about 10 - 50 than those from oceanic origins, i.e. European anthropogenic benzene stays in Europe and is transported over long distances within Europe.

High concentrations from the North Sea may result from oil drilling emissions.

Anthropogenic benzene of North America is unlikely to reach Europe by long-range transport over the Atlantic ocean.

**Source:** German Rapporteur

**Test condition:** Temperature-programmed gas chromatograph

**Test substance:** No data  
**Flag:** Risk Assessment  
 16-OCT-2002 (134)

**Memo:** Uptake of benzene by human beings.

**Result:** Daily intake of benzene by an adult as a function of the kinds and durations of exposure:

Exposure situation:	outdoor	indoor	inside a car	food
Concentration ( $\mu\text{g}/\text{m}^3$ ):	7	4	50	--
Duration of exposure (h):	2	21	1	--
Breath volume during exposure ( $\text{m}^3$ ):	1.7	17.5	0.8	--
Resorption rate (%):	50	50	50	100
Uptake ( $\mu\text{g}/\text{d}$ ):	6	35	20	5
Percentage in terms of total uptake (%):	9	53	30	8

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
 16-MAR-2004 (378)

**AQUATIC ORGANISMS****4.1 Acute/Prolonged Toxicity to Fish**

**Type:** flow through  
**Species:** Clupea harengus (Fish, estuary, marine)  
**Exposure period:** 6 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC :** = .7 -

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** A decrease in the survival of ovarian eggs, embryo and larvae was noted.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** Adult males and females (Clupea harengus pallasii) were exposed in saltwater with a dissolved oxygen concentration of 6-10.5 mg/l, a salinity of 23-24 g/l and a temperature of 10-11.5 degree C.  
**Reliability:** (2) valid with restrictions  
 16-MAR-2004 (1097)

**Type:** flow through  
**Species:** Morone saxatilis (Fish, estuary, marine)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 9.58 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Remark:** Comparable to guideline study  
 Effect values are related to measured concentrations.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** Juvenile fish (1-2g) were exposed in filtered and aerated natural saltwater (salinity: 28-30 g/l) at 16.9-17.9 degree C, alkalinity 110-120 mg/l as CaCO<sub>3</sub>, dissolved oxygen 7.2-8.2 mg/l and pH 7.6-7.8.  
**Test substance:** Reagent grade benzene was used.  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
 17-MAR-2004 (770)

**Type:** flow through  
**Species:** Mugil curema (Fish, estuary, marine)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC :** = 10 -

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Oxygen consumption was increased in a concentration-dependent manner to ca. 20-fold of the control value at 10 mg/l benzene. At the same concentration, the excretion of ammonia was reduced to ca. 60% of control. Significant methodological deficiencies. No analytical monitoring. According to the authors, benzene solutions were "emulsified".

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Juvenile white mullet (0.5-1.0 g) were captured from the wild and kept at 18-22 degree C in filtered, well-aerated seawater (salinity: 35 g/l) for 10 days prior to the assay. Fish were fed ad libitum. Oxygen consumption rates of individual fish (7 replicates) were recorded for 24 h during exposure to 1.0, 5.0, and 10.0 mg/l benzene, respectively. No further details were available. In parallel, ammonia excretion rates were monitored in a static system.

**Reliability:** (3) invalid  
02-SEP-2003 (245)

**Type:** flow through  
**Species:** Oncorhynchus kisutch (Fish, fresh water, marine)  
**Exposure period:** 1 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50 :** 1.75 -

**Method:** other  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Remark:** At 1.9 mg/l, about half the expected number of animals was found in the test arm with treated water. Nearly all fish avoided water with 2.8 mg/l benzene. No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail. Only 2 concentrations tested.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Presmolt coho salmon (6-10 cm length), kept in freshwater, were treated at 6.7-9.9 degree C with three different concentrations of benzene in a flow-through system. Avoidance reaction was tested in a Y-maze chamber with one

side continuously perfused with freshwater, the other side with freshwater containing benzene which was added from a reservoir by a syringe pump. 20-60 fish were placed in the holding region of the Y-maze for 15 min before injection of benzene to the water of one arm was started. After 50-70 min, the number of animals in each of the two upstream arms of the Y-maze was counted. Analytical monitoring showed that measured concentrations of test substance well agreed with calculated concentrations. The calculated EC50 value represents the concentration of benzene at which half the expected number of fish would be found in the test arm with treated water.

**Reliability:** (2) valid with restrictions  
16-MAR-2004 (749)

**Type:** flow through  
**Species:** Oncorhynchus mykiss (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 5.3 -

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Method:** Several parameters were not reported in the study (purity of test substance, EC0, EC100 values).

**Remark:** Test procedure in accordance with national standard methods with acceptable restrictions.  
Effect values are related to measured concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association, New York, 1975 was followed. Groups of 20 juvenile fish (*Salmo gairdneri*/*Oncorhynchus mykiss*, mean weight 13.9 g) were exposed to benzene in freshwater at 13-17 degree C. Dissolved oxygen concentration was about 7 mg/l, pH was 7.9-8, alkalinity 147-165 mg/l as CaCO<sub>3</sub> and water hardness 535-596 mg/l as CaCO<sub>3</sub>. The fish were not fed during treatment. The photoperiod was 16 h light/ 8 h dark. Benzene concentrations in the test tanks were monitored daily by HPLC.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (287)

**Type:** flow through  
**Species:** Oncorhynchus mykiss (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 21.6 -

**Method:** other  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Remark:** Comparable to guideline study with acceptable restrictions. Effect values are related to measured concentrations.

**Result:** In the same study, under similar conditions the toxicity of benzene to rainbow trout was also investigated after i. p. injection (solution in cod liver oil) and after oral administration (intubation of substance in gelatine capsules).  
i.p. injection (ten fish weighing 9-30 g each): LD50 value 25.8 mmol/kg (=2.0 g/kg fish).  
oral administration (ten fish weighing 9-30 g each): LD50 value 32.3 mmol/kg (=2.5 g/kg fish).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish (1-4g weight/fish), preadapted for at least one week, were exposed to benzene in a continuous-flow test system. Each bioassay tank contained 14 l of water. The tanks were not aerated during exposure. Water temperature was 14.1-16.5 degree C. Water was dechlorinated by charcoal filtration and addition of 1 mg/l sodium sulfite. Alkalinity averaged 86 mg/l (as CaCO3), conductivity 340 umhos/cm2, pH 7.6-8.2, oxygen content 5.6-9.4 mg/l. Benzene concentrations were measured daily by UV-spectrophotometry.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (512)

**Type:** flow through  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 15.6 -

**Method:** other  
**Year:** 1992  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Comparable to guideline study.

**Result:** Effect values related to measured concentrations.  
LC50 confidence limit = 14.04-17.3 mg/l.

**Source:** In the same study a 7d-LC50 of 14.02 mg/l and a 7d-NOEC of 10.02 mg/l are reported for the endpoints growth and survival.  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of the U.S. EPA (Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 2nd Edition. EPA 600/4-89-001 and Supplement EPA 600/4-89-001A, Cincinnati, Ohio, 1989) was followed. Chemical analysis was conducted by GC-FID. Groups of 40 fish aged less than 24 hours were exposed to benzene in filtered, aerated and heated Lake Superior water. The temperature was measured to be 24-26 degree C, pH 7.1-8.3, dissolved oxygen 6.3-8.3 mg/l, alkalinity 38-40 mg/l as CaCO3 and hardness 44.5-46.5 mg/l as CaCO3. The photoperiod was 16 h light/ 8 h dark. Fish were fed three times a day with brine shrimp larvae. The benzene concentrations in the tanks were measured before starting tests and at least twice during the test.

**Test substance:** Supplied by Burdick & Jackson, Muskegon, Midland; >99.5% pure.

**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment

17-MAR-2004 (743)

**Type:** flow through  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC30 :** 15.1 -

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Method:** Several parameters were not reported in the study (purity of test substance, EC0, EC100 values).  
**Remark:** Test procedure in accordance with national standard methods with acceptable restrictions.  
 Effect values are related to measured concentrations.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 14th Edition. American Public Health Association, New York, 1975 was followed. Groups of 20 juvenile fathead minnows (mean weight 0.58 g) were exposed to benzene in freshwater at 13-17 degree C. Dissolved oxygen concentration was about 7 mg/l, pH was 7.9-8, alkalinity 147-165 mg/l as CaCO3 and water hardness 535-596 mg/l as CaCO3. The fish were not fed during treatment. The photoperiod was 16 h light/ 8 h dark. Benzene concentrations in the test tanks were monitored daily by HPLC.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (287)

**Type:** semistatic  
**Species:** Oncorhynchus mykiss (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 5.9 -

**Method:** OECD Guide-line 203 "Fish, Acute Toxicity Test"  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Several experimental details not reported (age of fish, measured benzene concentrations, applied concentrations).  
 Effect values are related to measured concentrations.  
**Source:** BP Chemicals Ltd LONDON  
 German Rapporteur  
**Test condition:** Groups of ten fish were exposed to benzene in freshwater. The water temperature was 12°C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.  
**Test substance:** Supplied by Carlo Erba, purity not specified.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (391) (827)

**Type:** semistatic  
**Species:** Poecilia reticulata (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 28.6 -

**Method:** OECD Guide-line 203 "Fish, Acute Toxicity Test"  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Effect values related to measured concentrations.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish were exposed, in freshwater, to benzene (11 l closed bottles, 10 animals/10 l, renewal of test solution after 48 h to avoid decline of oxygen saturation below 80%).  
The water temperature was 21 degree C. The lethal effect was observed at 3, 6, 24, 48, 72 and 96 hours from the beginning of the experiment.  
Quantitative analyses of benzene were carried out by HPLC at the beginning and the end of the experiment, and at the renewal of the test solution after 48 h.

**Test substance:** purity: Carlo Erba GC standards.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (391)

**Type:** semistatic  
**Species:** Poecilia reticulata (Fish, fresh water)  
**Exposure period:** 14 day(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** = 63.5 -

**Method:** other  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of eight fish aged 2-3 months were exposed to benzene in 1 l of standard freshwater in 1.5 l vessels covered with glass. Water hardness was 25 mg/l as CaCO<sub>3</sub>, oxygen content remained about 5 mg/l and temperature was 21-23 degree C. The test solution was renewed daily.  
Benzene was added as stock solution in acetone or isopropanol.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (627)

**Type:** static  
**Species:** Brachydanio rerio (Fish, fresh water)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**NOEC:** < 32 -  
**LC50:** 70.7 -

**Method:** other: according to UBA guideline of March 1979 and addendum of 06.02.1980  
**Year:** 1980  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Source:** BASF AG Ludwigshafen  
German Rapporteur  
**Reliability:** (2) valid with restrictions  
16-MAR-2004 (76)

**Type:** static  
**Species:** Carassius auratus (Fish, fresh water)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** 46 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** Test procedure in accordance with national standard methods with acceptable restrictions  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater, Method No. 231, 1971, was followed. Groups of six fish (2.3-4.3 g) were exposed to benzene at 19-21 degree C in 25 l tap-water (100 mg/l Ca<sup>2+</sup>, alkali as Na<sup>+</sup>: 30 mg/l, pH 7.8, no aeration during exposure to reduce evaporation of benzene, dissolved oxygen during test at least 4mg/l).  
The concentration of the test chemical was determined before and after each test.  
**Test substance:** Purity: no data presented.  
**Reliability:** (2) valid with restrictions  
17-MAR-2004 (144)

**Type:** static  
**Species:** Carassius auratus (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** = 34.42 -

**Method:** other  
**Year:** 1966  
**GLP:** no data  
**Test substance:** other TS: reagent grade

**Remark:** Concentration of benzene was not checked analytically in an open, static test system (wide-mouth bottles).  
**Result:** LC50 confidence limits = 26.10-42.83 mg/l.  
 Further tests gave 48- and 96-hour LC50 values of 34.42 mg/l (confidence limits 26.10-42.83 mg/l).  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25 degree C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).  
**Test substance:** Reagent grade benzene tested.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
 17-MAR-2004 (895)

**Type:** static  
**Species:** Clupea harengus (Fish, estuary, marine)  
**Exposure period:** 7 day(s)  
**Unit:** µg/l **Analytical monitoring:** no data  
**EC :** = 53 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** A significant decrease in oxygen consumption was recorded at the lower concentration tested, ca. 50 ug/l (0.06 µl/l), while the higher concentration, ca. 500 ug/l (0.56 µl/l), caused a significant increase in oxygen consumption. No effects on anabolic energy, tissue weight or growth were found.  
  
 No effects on survival or yolk consumption were seen at both concentrations tested, 500 ug/l being the higher. A significant decrease in tissue weight and significant delayed growth were seen both in those exposed to 50 and 500 ug/l.  
 Documentation insufficient for assessment  
 Concentration of test substance not exactly defined.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** Embryo (Clupea harengus pallasi) were exposed, at hatching,

to a single dose of benzene at the beginning of the exposure period in saltwater with a salinity of 23 g/l and a temperature of 13.5 degree C.

**Reliability:** (3) invalid  
02-SEP-2003 (327)

**Type:** static  
**Species:** Clupea harengus (Fish, estuary, marine)  
**Exposure period:** 12 day(s)  
**Unit:** µg/l **Analytical monitoring:** no data  
**NOEC:** = 1850 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Concentration of test substance not exactly defined.  
In post-yolk sac larvae similarly exposed for 12 days at 12.2°C, a small increase in growth and caloric assimilation was noted at 120 and 1850 ug benzene/l.  
A significant effect on oxygen consumption was noted at 120 and 1850 ug/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Post-yolk sac larvae (Clupea harengus pallasii) were exposed in saltwater with a salinity of 23 g/l and a temperature of 12.2°C.

**Reliability:** (3) invalid  
16-MAR-2004 (327)

**Type:** static  
**Species:** Clupea harengus (Fish, estuary, marine)  
**Unit:** µg/l **Analytical monitoring:** no data  
**LC0:** = 460 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Concentration of test substance not exactly defined.  
No effects on survival, yolk consumption or catabolic energy were seen at 460 ug/l, the highest concentration tested. A significant energy deficit, however, was reported at this concentration when the larvae were tested 24 hours after hatching.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Yolk sac larvae (Clupea harengus pallasii) were exposed for 5 days to benzene in saltwater at 12.5 degree C and a salinity of 23 g/l.

**Reliability:** (3) invalid  
16-MAR-2004 (327)

**Type:** static  
**Species:** Gasterosteus aculeatus (Fish, estuary, marine)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 21.8 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Result:** LC50 95% confidence limits = 19-25 mg/l.  
 Most deaths occurred during the first 12 h of exposure.  
 Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Three-year-old adult three spine sticklebacks (length ca. 55 mm, 12 fish/concentration) were exposed in freshwater at 8 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen content >85% saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** purity not specified  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (787)

**Type:** static  
**Species:** Ictalurus punctatus (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 425 -

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** LC50 confidence limits = 357-505 mg/l.  
 Further 96h LC50 values (confidence limit) given:  
 rainbow trout (appr. 2.4g), 12 degree C: 9.2 mg/l (7.2-11.7)  
 Bluegill sunfish (appr. 0.2 g), 22 degree C: 100 mg/l (70-142).  
 Secondary literature  
 Data from handbook with compilation of results

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Fish (approx. 0.1 g) exposed in freshwater aerated prior to use at 22 degree C to benzene. Water hardness was 40-50 mg/l as CaCO<sub>3</sub>, alkalinity was 30-35 mg/l as CaCO<sub>3</sub> and pH was 7.2-7.5.

**Reliability:** (4) not assignable  
 02-SEP-2003 (585)

**Type:** static  
**Species:** Lepomis macrochirus (Fish, fresh water)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** = 20 -  
**LC100:** = 34 -

**Method:** other  
**Year:** 1954  
**GLP:** no data  
**Test substance:** no data

**Remark:** No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
 No monitoring of concentration during the test, purity of test substance not reported.

**Result:** A 48 h LC50 of 20 mg/l was also reported in this study.  
 At 60 mg/l, all fish died within two hours.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Groups of ten bluegill sunfish (length: 5-11 cm, weight ca. 5 g) were exposed for 48 h to benzene in 15 l freshwater (alkalinity as CaCO<sub>3</sub>: 33-81 mg/l, pH 6.9-7.5, total hardness as CaCO<sub>3</sub>: 84-163 mg/l, dissolved oxygen at least 5 mg/l). Benzene was added from a saturated solution in water. The concentration in this solution was determined by UV-spectrophotometry.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
 17-MAR-2004 (1170)

**Type:** static  
**Species:** Lepomis macrochirus (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 22.49 -

**Method:** other  
**Year:** 1960  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Concentration of benzene was not checked analytically in an open, static test system (wide-mouth bottles).

**Result:** LC50 confidence limits = 17.50-28.37 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25 degree C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).

**Test substance:** Reagent grade benzene tested.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment

17-MAR-2004

(895)

**Type:** static  
**Species:** Morone saxatilis (Fish, estuary, marine)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 6.1 -

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Similar exposure for 96 hours gave an LC50 value of 5.8 ppm (converts to 5.1 mg/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Juveniles (mean weight: 6.0 g) were exposed at 16 degree C in saltwater (salinity: 25 g/l). Benzene concentration was assayed by gas chromatography at 0, 24, 48, 72, and 96 hours.

**Test substance:** >99% pure.  
**Reliability:** (2) valid with restrictions

16-MAR-2004

(94)

**Type:** static  
**Species:** Mugil curema (Fish, estuary, marine)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** = 22 -

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Significant methodological deficiencies  
No analytical monitoring, no EC0 value, no confidence limits reported, no. of animals not reported. According to the authors, benzene solutions were "emulsified".

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Juvenile white mullet (0.5-1.0 g) were captured from the wild and kept at 18-22 degree C in filtered, well-aerated seawater (salinity: 35 g/l) for 10 days prior to the assay. Fish were fed ad libitum.  
48 h LC50 bioassays were conducted in 2.5 l glass bottles with water aerated before benzene was added from stock solution (1000 mg/l).

**Reliability:** (3) invalid

16-MAR-2004

(245)

**Type:** static  
**Species:** Oncorhynchus gorbuscha (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 15 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 12.6-17.4 mg/l.  
 Most deaths occurred during the first 12 h of exposure.  
 Fry acclimated to saltwater (salinity 28-30 g/l) similarly exposed to benzene were more sensitive: LC50 value 7.4 mg/l (confidence limits 6.9-8 mg/l).  
 When examining eggs and emergent fry in freshwater, LC50 values of 298 and 4.6 mg/l were obtained, respectively.  
 Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Outmigrant Pink salmon fry were exposed at 4 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure >85% dissolved oxygen saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** purity not specified  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (787)

**Type:** static  
**Species:** Oncorhynchus kisutch (Fish, fresh water, marine)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 12.4 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 8.7-16.1 mg/l.  
 Most deaths occurred during the first 12 h of exposure.  
 This test was repeated using eggs and emergent fry. The 96-hour LC50s were 476 and 8.6 mg/l respectively.  
 Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Coho salmon (length 5-50 mm) were exposed to benzene at 9 degree C in freshwater. Solutions were aerated during the

final 48 h to ensure >85% dissolved oxygen saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** Purity not specified  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (787)

**Type:** static  
**Species:** Oncorhynchus nerka (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 9.5 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 8.3-10.6 mg/l.  
Most deaths occurred during the first 12 h of exposure. Smolts acclimated to saltwater (salinity 28-30 g/l) similarly exposed to benzene were more sensitive: LC50 value 4.9 mg/l (confidence limits 2.5-7.2 mg/l).  
Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Sockeye salmon smolts were exposed at 6 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure >85% dissolved oxygen saturation. Concentration of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** Purity not specified.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (788)

**Type:** static  
**Species:** Oncorhynchus tshawytscha (Fish, fresh water, marine)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 10.3 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 7.4-12 mg/l.  
 Most deaths occurred during the first 12 h of exposure.  
 Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Juvenile chinook salmon (length 75 mm) were exposed (12 fish/concentration) at 9 degree C to benzene in freshwater. Solutions were aerated during the final 48 h to ensure dissolved oxygen content >85% saturation. Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** Purity not specified  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (788)

**Type:** static  
**Species:** Oryzias latipes (Fish, fresh water)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** 54 - 74

**Method:** other  
**Year:** 1986  
**GLP:** no data  
**Test substance:** no data

**Remark:** At 10, 20 and 30 degree C, the 24 h as well as the 48 h LC50s were 74, 70 and 54 mg/l respectively. Other investigators reported a 48-hour LC50 of 250 mg/l in fish exposed at 4-5 weeks of age to benzene (>98% pure) at 24°C (Slooff W. et al. Aquat. Toxic. 4, 113-128, 1983, cited in AQUIRE, 1993).  
 Original reference in Japanese

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Killifish were exposed to benzene in freshwater at various temperatures to examine the effect of temperature on the LC50 values.

**Reliability:** (4) not assignable  
 16-MAR-2004 (1163)

**Type:** static  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** 34.42 - 35.56

**Method:** other  
**Year:** 1960  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Concentration of benzene was not checked analytically in an open, static test system (wide-mouth bottles).

**Result:** Lower value for test in hard water;  
LC50 confidence limits = 26.10 - 42.83 mg/l.  
Higher value for test in soft water;  
LC50 confidence limits = 25.81 - 45.83 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene in either soft or hard freshwater at 25 degree C. The hardness was measured as 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5-8.2 and dissolved oxygen 7.8 mg/l.

**Test substance:** Reagent grade benzene tested.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
16-MAR-2004 (895)

**Type:** static  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** 32 - 35.08

**Method:** other  
**Year:** 1960  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Concentration of benzene was not checked analytically in an open, static test system (wide-mouth bottles).

**Result:** Lower value for test in hard water;  
LC50 confidence limits = 22.33 - 41.16 mg/l.  
Higher value for test in soft water;  
LC50 confidence limits = 26.74 - 43.67 mg/l.

Further results over 96 hours were reported:  
In soft water: 33.47 mg/l (95 % CL: 25.19 - 41.00 mg/l);  
In hard water: 32.00 mg/l (95 % CL: 22.33 - 41.10 mg/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of

ten fish were exposed to benzene in either soft or hard freshwater at 25 degree C. The hardness was measured to be 20 and 360 mg/l (EDTA) respectively, alkalinity 18 and 300 mg/l respectively, pH 7.5-8.2 and dissolved oxygen 7.8 mg/l.

**Test substance:** Reagent grade benzene tested.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
17-MAR-2004 (895)

**Type:** static  
**Species:** *Poecilia reticulata* (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC50:** = 36.6 -

**Method:** other  
**Year:** 1966  
**GLP:** no data  
**Test substance:** other TS: reagent grade

**Remark:** Concentration of benzene was not checked analytically in an open, static test system (wide-mouth bottles).  
Test procedure in accordance with national standard methods with acceptable restrictions  
Synonym for the species tested: *Poecilia reticulata*.  
LC50 confidence limits = 28.63-54.33 mg/l.  
Further tests gave 48- and 96-hour LC50 values of 36.60 mg/l (confidence limits 28.63-54.33 mg/l).

**Source:** German Rapporteur  
**Test condition:** The method of the APHA. Standard Methods for the Examination of Water and Wastewater. 11th Edition. American Public Health Association, New York, 1960 was followed. Groups of ten fish were exposed to benzene at 25 degree C in freshwater (hardness 20 mg/l (EDTA), alkalinity 18 mg/l, pH 7.5 and dissolved oxygen 7.8 mg/l).

**Test substance:** Reagent grade benzene tested.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
17-MAR-2004 (895)

**Type:** static  
**Species:** Salmo trutta (Fish, fresh water, marine)  
**Exposure period:** 1 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 12 -

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The method employed in this study developed, with certain modifications, into the standard method to be used in conditions of consent for effluents, by the Ministry of Housing and Local Government. Fish Toxicity Tests. Report of the Technical Committee. HMSO, London, 1969. Groups of ten yearling fish were exposed to benzene at 10 degree C in freshwater.

**Reliability:** (2) valid with restrictions  
 16-MAR-2004 (1278)

**Type:** static  
**Species:** Salvelinus malma (Fish, marine)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 10.5 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LD50 confidence limits = 7.9-13 mg/l.  
 Most deaths occurred during the first 12 h of exposure. Smolts acclimated to saltwater (salinity 28-30 g/l) were more sensitive to benzene (LC50 value 5.5 mg/l, 95% confidence limits 4-7 mg/l) than smolts in freshwater (LC50 value 10.5 mg/l, confidence limits 7.8-13.2 mg/l). Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Dolly varden were exposed to benzene (12 fish/concentration) in freshwater at 8 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen content at >85% of the saturation level.  
 Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96h, respectively.

**Test substance:** Purity not specified  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (787)

**Type:** static  
**Species:** other: Cottus cognatus (slimy sculpin)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 13.5 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 12.2-15 mg/l.  
Most deaths occurred during the first 12 h of exposure.  
Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Cottus cognatus (slimy sculpin) yearlings (length 50 mm) were exposed in freshwater at 9 degree C. Solutions were aerated during the final 48 h to ensure dissolved oxygen levels at >85% of saturation concentration.  
Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** Purity not specified.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (787)

**Type:** static  
**Species:** other: Thymallus arcticus (Arctic grayling)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 12.9 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** no data

**Remark:** No EC0, EC100 reported, purity of test substance not specified.

**Result:** LC50 confidence limits = 9.4-16.5 mg/l.  
Most deaths occurred during the first 12 h of exposure.  
Effect values are related to initial nominal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Thymallus arcticus (Arctic grayling) yearlings (length 55 mm) were exposed in freshwater at 9 degree C to benzene. Solutions were aerated during the final 48 h to ensure dissolved oxygen content at >85% saturation.  
Concentrations of benzene as monitored by UV-spectrophotometry (at 254 nm) decreased to ca. 75% of initial value in 24 h and to ca. 10% after 96 h, respectively.

**Test substance:** Purity not specified.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (787)

**Type:** other  
**Species:** Clupea harengus (Fish, estuary, marine)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** 20 - 25

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Remark:** A second study was conducted in which eggs were exposed in saltwater for 96 hours at 10-17.5 degree C. The LC50 was 40-45 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Two-day-old larvae (Clupea harengus pallasii) were exposed in a renewal type assay in saltwater to benzene at 11.5-14.0 degree C. The water pH was 7.4-8.1 and dissolved oxygen content was 4.7-8.0 mg/l. The larvae were observed for 7 days after exposure.

**Reliability:** (4) not assignable  
16-MAR-2004 (1095)

**Type:** other  
**Species:** Cyprinus carpio (Fish, fresh water)  
**Exposure period:** 7 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC0:** = 1500 -

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Fish were exposed in freshwater at 17-18 degree C and pH 7.1-8.1.

**Reliability:** (4) not assignable  
16-MAR-2004 (39) (381)

**Type:** other  
**Species:** Gambusia affinis (Fish, fresh water)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LC50:** = 395 -

**Method:** other  
**Year:** 1957  
**GLP:** no data  
**Test substance:** other TS

**Remark:** After exposure for 48 and 96 hours, the LC50 values were found to be 395 and 386 mg/l respectively. At 300 mg/l and less, the fish were apparently normal and at 1000 mg/l all were dead within 16 min.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish were exposed to benzene in pond water which had a long history of high turbidity. The alkalinity was low (10 ppm methyl orange alkalinity), pH ranged from 8.1-8.4 and temperature from 20-22 degree C.

**Test substance:** Chemically pure benzene tested.

**Reliability:** (4) not assignable

16-MAR-2004

(1227)

**Type:** other  
**Species:** Oncorhynchus mykiss (Fish, fresh water)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC0:** = 40 -  
**LC50:** = 56 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish (Salmo gairdneri=Oncorhynchus mykiss, 5-8 weeks of age) were exposed to benzene in a total volume of 10 l tap water (hardness 5.5 degree d.H. corresponding to 98 mg CaCO3) at 15 degree C and pH 7.8.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (3) invalid

17-MAR-2004

(1052)

**Type:** other  
**Species:** Oryzias latipes (Fish, fresh water)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC0:** = 126 -  
**LC50:** = 250 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish (4-5 weeks of age) were exposed to benzene in a total volume of 1 l water (Dutch Standard Water) at 24 degree C.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (3) invalid  
17-MAR-2004 (1052)

**Type:** other  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**LC0:** = 54 -  
**LC50:** = 84 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten fish (3-4 weeks of age) were exposed to benzene in a total volume of 1 l water (Dutch Standard Water) at 20 degree C.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (3) invalid  
17-MAR-2004 (1052)

**Type:** other  
**Species:** Pimephales promelas (Fish, fresh water)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** = 12.6 -

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** LC50 confidence limits = 10.7-14.7 mg/l. Affected fish lost schooling behaviour, swam in a corkscrew motion, were underreactive to external stimuli and had increased respiration and convulsions. They were also darkly coloured and were haemorrhaging. Loss of equilibrium was seen prior to death.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Chemical analysis was conducted by GLC. Groups of ten fish were exposed at 30 days of age to benzene in freshwater at 22.3 degree C, 7.3 mg dissolved oxygen/l, 50.7 mg/l as CaCO<sub>3</sub> (hardness), 42.5 mg/l as CaCO<sub>3</sub> (alkalinity) and pH 7.0.

**Test substance:** Supplied by J.T. Baker Chemical Co.; >=99.9% pure.  
**Reliability:** (1) valid without restriction  
18-MAR-2004 (397)

**Type:** other  
**Species:** other: Engraulis mordax (Northern anchovy)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LC50:** 20 - 25

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Engraulis mordax (Northern anchovy) fish eggs were exposed in a renewal type assay in saltwater at 16.8-18.4 degree C, pH 7.5-8.1 and dissolved oxygen concentration 6.9-7.6 mg/l. Observations were made on days 3 and 6.

**Reliability:** (4) not assignable  
16-MAR-2004 (1096)

**4.2 Acute Toxicity to Aquatic Invertebrates**

**Species:** Artemia salina (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 66 -

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Result:** The 48-hour LC50 was also determined and found to be 21 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The method of Tarzwell C.M. (Standard Methods for Determination of Relative Toxicity of Oil Dispersants and Mixtures of Dispersants and Various Oils to Aquatic Organisms, Proc. Joint Conf. on Prevention and Control of Oil Spills, sponsored by API and EPA, December 15-17, 1969) was followed. A preliminary study was conducted to determine the range in which benzene was toxic to Artemia salina. 30-50 newly hatched brine shrimps were exposed in 100 ml artificial seawater for 24 hours at 24.5 degree C in loosely capped bottles. At the end of exposure, the number of live and dead shrimp were noted. Benzene was found to be lethal at less than 100 mg/l. The median tolerance limit was then examined at doses established from the preliminary screen.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
 17-MAR-2004

(910)

**Species:** Artemia sp. (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC50:** 127.3 -

**Method:** other  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In the study, the EC50 value is listed as 1630 mmol/m3. Significant methodological deficiencies  
 No analytical monitoring, concentrations calculated from solubility data.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Artemia were exposed to benzene at 19-21 degree C in test chambers sealed in glass vials with teflon-lined screw caps. Benzene was added as saturated solution in water (lower phase of water/benzene mixture in a separatory funnel). The concentration of the stock solution was calculated from solubility data and it was assumed that loss of benzene due to volatilisation was negligible.  
 No analytical monitoring was performed.

No further details are reported in this study.

**Test substance:** Purity: at least 97% (no further specification)

**Reliability:** (3) invalid (5)

17-MAR-2004

**Species:** Asellus sp. (Crustacea)

**Exposure period:** 48 hour(s)

**Unit:** mg/l **Analytical monitoring:** no data

**EC50:** = 120 -

**Method:** other

**Year:** 1983

**GLP:** no data

**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 Asellus aquaticus (Isopoda) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.

The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

17-MAR-2004 (1049)

**Species:** Daphnia cucullata (Crustacea)

**Exposure period:** 48 hour(s)

**Unit:** mg/l **Analytical monitoring:** no data

**EC50:** 356 - 390

**Method:** other

**Year:** 1978

**GLP:** no data

**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Test followed the concept rules of the Dutch Standardization Institute (1978), but no experimental details are reported in this study (no. of animals, temperature, open or closed system).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Daphnids were exposed at 10-12 days of age in static freshwater. The actual concentrations of the test compound during the experiments were not measured and LC50 values were calculated from the quantity added at the start.

**Reliability:** (4) not assignable

17-MAR-2004 (174)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**IC50 :** = 18 -

**Method:** OECD Guide-line 202  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Effect values are related to measured concentrations.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The determination of the immobilization concentration for 50% of the organisms was performed in closed bottles (150 ml), completely filled with test solution.

**Test substance:** purity: Carlo Erba GC standard.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (391)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC50:** = 10 -

**Method:** OECD Guide-line 202  
**Year:** 1993  
**GLP:** no data  
**Test substance:** other TS

**Remark:** No information whether closed systems were used.  
**Result:** According to the authors, test procedure followed OECD guideline 202, part 1, but details not reported in the study.  
After 24 h of exposure, the EC50 was also 10 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** Purity: reagent grade  
**Reliability:** (4) not assignable  
**Flag:** Risk Assessment  
17-MAR-2004 (579)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 1 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC50:** = 6.3 -

**Method:** other  
**Year:** 1993  
**GLP:** no data  
**Test substance:** other TS

**Remark:** According to the authors, results of this test correlate well with tests done in parallel following OECD-guideline criteria.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The test is based on the ability of the alive animals to enzymatically hydrolyse 4-methylumbelliferyl-beta-D-galactoside (MUF-G) with the formation of fluorescent 4-methylumbelliferon inside the animal.  
 Toxicity tests were run as follows:  
 five benzene concentrations plus control,  
 six 10 ml glass test tubes with five 12-48 h old Daphnia magna per treatment,  
 incubation for 1 h at 20 degree C,  
 addition of 0.1 ml of MUF-G stock solution (5 g/l),  
 corresponding to 50 mg/l,  
 counting of fluorescent animals 15 min later under UV light.  
 Tests were considered valid if at least 90% of control animals showed fluorescence.

**Test substance:** Purity: Reagent grade  
**Reliability:** (2) valid with restrictions  
 17-MAR-2004 (579)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 250 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** other TS

**Remark:** LC50 confidence limits = 200-310 mg/l. The no discernible effect concentration was <13 mg/l.  
 Original reference not available

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009), 1975 was used. Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO<sub>3</sub>, pH of 7.4-9.4, dissolved oxygen concentration of 6.5-9.1 mg/l and a temperature of 22 degree C. Dilution water was used for the negative control.

**Test substance:** >= 80% pure.  
**Reliability:** (4) not assignable  
17-MAR-2004 (673)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC50:** 31.2 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** 95% confidence interval: 22.2-43.3 mg/l.  
In the study, values are presented as mmol/m3.  
Significant methodological deficiencies  
No analytical monitoring, applied concentrations of test  
substance calculated from solubility data.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Daphnia (4-6 days old) were exposed to benzene in distilled  
water as follows:  
10 animals in 24 ml glass vials with Teflon-lined screw caps  
completely filled,  
5 concentrations of benzene and control,  
temperature: 21-25 degree C,  
pH ca. 7 at start and ca. 6 at end of exposure  
dissolved oxygen ca. 9 mg/l at start and 5 mg/l at end of  
exposure.  
Benzene was added as saturated stock solution in water  
(lower phase of water/benzene mixture in a separatory  
funnel). The concentration of the stock solution was  
calculated from solubility data and it was assumed that loss  
of benzene due to volatilisation was negligible.  
No analytical monitoring was performed.

**Test substance:** Purity: at least 97% (no further specification)  
**Reliability:** (3) invalid  
17-MAR-2004 (121)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 200 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** other TS

**Remark:** LC50 confidence limits = 140-320 mg/l. The no discernible effect concentration was <13 mg/l.  
Original reference not available

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The method of the US EPA. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series (EPA-660/3-75-009), 1975 was followed. Groups of 15 daphnids were exposed to benzene in reconstituted well water having a total hardness of 173 mg/l as CaCO<sub>3</sub>, pH 7.4-9.4, dissolved oxygen concentration of 6.5-9.1 mg/l and a temperature of 22 degree C. Dilution water was used for the negative control.

**Test substance:** >= 80% pure.  
**Reliability:** (4) not assignable  
17-MAR-2004 (39) (674)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** 356 - 620

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
According to the authors, test followed the concept rules of the Dutch Standardization Institute (1978), but no experimental details are reported in this study (no. of animals, temperature, open or closed system).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Daphnids were exposed at <1 day of age in static freshwater. The actual concentrations of the test compound during the experiments were not measured and LC50 values were calculated from the quantity added at the start.

**Reliability:** (4) not assignable  
17-MAR-2004 (174)

**Species:** Daphnia magna (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** 682 -

**Method:** other  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** 95% confidence limit: 608-752 mg/l.  
No. of animals not exactly reported, no ECO, EC100 values,  
no analytical monitoring, incubation conditions not  
precisely described (open or closed system).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 5 or 10 animals (no further information) were treated in  
Petri dishes (no information, if open or closed) with  
various concentrations of benzene in filtered, autoclaved  
river water (alalinity as CaCO<sub>3</sub>: 92 mg/l, hardness as CaCO<sub>3</sub>:  
134, pH 7.4-7.8, oxygen: 3.8-4.0, temperature: 17.5-20.5  
degree C). Acetone was used as carrier (0.5 ml/l) and  
Triton-X100 as a surfactant (0.5 g/l) in all tests including  
controls.

**Test substance:** Benzene from a commercial supplier was used without further  
purification (No additional data given).

**Reliability:** (3) invalid  
17-MAR-2004

(315)

**Species:** Daphnia pulex (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** 265 - 345

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
According to the authors, test followed the concept rules of  
the Dutch Standardization Institute (1978), but no  
experimental details are reported in this study (no. of  
animals, temperature, open or closed system).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Daphnids were exposed at <1 day of age in static freshwater.  
The actual concentrations of the test compound during the  
experiments were not measured and LC50 values were  
calculated from the quantity added at the start.

**Reliability:** (4) not assignable  
17-MAR-2004

(174)

**Species:** Daphnia pulex (Crustacea)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** = 15 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Remark:** Effect values are related to measured concentrations.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 20 daphnids per concentration were exposed in filtered lake water at 15 degree C (13-17 degree C) and pH 7.5. Sealed 250 ml Erlenmeyer flasks with sufficient head space to accomodate in excess the oxygen demand were used. Benzene was tested at concentrations ranging from 0 to 20 mg/l. Benzene concentrations were determined by UV-spectrofluorometry.

**Test substance:** purity: no data  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1160)

**Species:** Daphnia pulex (Crustacea)  
**Exposure period:** 1 day(s)  
**Unit:** mg/l **Analytical monitoring:**  
**EC :** = 50 -

**Year:** 1985  
**GLP:** no data  
**Test substance:** no data

**Remark:** In a field study in static freshwater, with a dissolved oxygen concentration of 1.1-1.6 mg/l, pH of 6.2, a temperature of 12 degree C and a hardness of 2.86 mmol/l, the lower concentration tested, 50 mg/l, was lethal to Daphnia pulex exposed for <1 day. Benzene loss and oxygen fluctuations were reported.  
Significant methodological deficiencies  
Near 100% lethality at lower dose tested, no concentration-dependent effects on lethality investigated.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (3) invalid  
17-MAR-2004 (670)

**Species:** Gammarus pulex (Crustacea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 42 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 Gammarus pulex (Amphipoda) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.

The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

17-MAR-2004

(1049)

**Species:** Nitocra spinipes (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** = 82 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Result:** 95% confidence limit: 73-91 mg/l.  
At increased salinity (25 g/l), the EC50 value was 111.5 mg/l (conf. lim. 61-157 mg/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** According to the author, the bioassay followed methods outlined in Standard Methods (APHA, 1965).  
10-20 Nitocra spinipes (Harpacticoida, Copepoda, lab culture from wild captures), preadapted without food to test conditions for 12 h, were exposed to benzene under the following conditions:

150 ml fingerling bowls, salinity: 15 g/l, oxygen concentration: at least  $\geq 5.8$  ppm at end of experiment, temperature: 20 degree C.

Effects of benzene on animals were recorded by microscopical observation.

Benzene concentrations were measured by UV-spectrophotometry (at 250 nm).

**Test substance:** Purity: ACS standard, redistilled.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

17-MAR-2004

(904)

**Species:** Palaemonetes pugio (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** = 33.5 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** other TS

**Result:** At different temperatures and salinities, the following results were obtained:

Stage	Temp. (degree C)	Salin. (g/l)	EC50 (mg/l)	95% confidence limit (mg/l)
Adult	20	15	38.0	36.6-40.2
	20	25	33.5	31.0-36.0
	10	15	40.2	33.5-48.2
	10	25	40.8	38.6-43.3
Larval	20	15	90.8	61.1-113.5
	20	25	74.4	66.8-81.8

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Bioassays were conducted with adult and larval brine shrimp, respectively.  
 13-25 adult shrimp (wild captures acclimated at least 10 days) were exposed in 10 l seawater to benzene at different temperatures and salinities (see below). Oxygen content was at least  $\geq 5.8$  ppm at the end of the experiment.  
 Approx. 30 larval shrimp (capture bred) were exposed in 350 ml glass bowls.  
 Benzene concentrations were measured by UV-spectrophotometry (at 250 nm).  
 Effects of benzene on animals were recorded by microscopical observation.

**Test substance:** Purity: ACS standard, redistilled.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

17-MAR-2004

(904)

**Species:** Palaemonetes pugio (Crustacea)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 43.5 -

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Remark:** When the same test was conducted over 48 or 96 hours, LC50 values of 35 and 27 mg/l were obtained respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
 German Rapporteur

**Test condition:** The shrimp were exposed under static conditions in saltwater which had a pH of 8.1, dissolved oxygen concentration of >5 mg/l, salinity of 15 g/l and a temperature of 21 degree C.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
 17-MAR-2004 (1109)

**Species:** other aquatic mollusc: Crassostrea gigas (Bivalvia)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 377 -

**Method:** other  
**Year:** 1974  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
 No information on several parameters (pH, monitoring of test substance, oxygen content).  
 ED50 value given refers to median ecological mortality (100 - Perc. Ecological Survival, which is the no. of surviving larvae in the culture minus the no. of abnormal larvae in culture).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Crassostrea gigas (Pacific oyster) fertilised eggs (ca. 30000/test container) were exposed in static saltwater at 20-21.5 degree C and at a salinity of 25.3-30.8 g/l.

**Reliability:** (4) not assignable  
 17-MAR-2004 (679)

**Species:** other aquatic mollusc: *Lymnaea stagnalis*  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC0:** = 120 -  
**EC50:** = 230 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten *Lymnaea stagnalis* were exposed at 3-4 weeks of age in 1 l of Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982) at 20 degree C in closed vessels.

**Test substance:** Purity: highest grade available (at least >98% pure, no further information given).

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1049)

**Species:** other aquatic worm: *Dugesia cf. lugubris* (Turbellaria)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 74 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Result:** In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 *Dugesia cf. lugubris* (triclad flatworm) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 °C.

The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1049)

**Species:** other aquatic worm: Limnodrilus and Tubifex spec.  
(Oligochaeta)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** > 320 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 Tubificidae were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.

**Test substance:** The animals were obtained from a commercial source.  
purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

17-MAR-2004

(1049)

**Species:** other aquatic crustacea: Cancer magister  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** 108 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Full details of the method not reported (open/closed system, oxygen content, pH).

**Result:** The concentration of benzene as measured by UV-spectrophotometry (at 255 nm) was found to decline by about 25-50% within the first 24 h of exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 50 first instar zoea larvae of the dungeness crab (Cancer magister) were exposed in static saltwater at 10.5-14.2 degree C and a salinity of 29-34 g/l.

**Reliability:** (4) not assignable  
**Flag:** Risk Assessment

17-MAR-2004

(172)

**Species:** other aquatic crustacea: Crangon franciscorum (bay shrimp)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 19.3 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** other TS

**Remark:** LC50 95% confidence limits = 17.6-21.1 mg/l.  
When conducted over 96 hours, the LC50 was found to be  
17.6 mg/l (confidence limit 16.7-19.3 mg/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 10 mature Crago franciscorum (bay shrimp) were exposed in  
saltwater at 16 degree C. The salinity was 25 g/l.

**Test substance:** >99% pure.

**Reliability:** (4) not assignable

17-MAR-2004

(94)

**Species:** other aquatic crustacea: Diaptomus forbesi (calanoid copepod)  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 710 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** A static freshwater system at 28 degree C was used to test  
Diaptomus forbesi (calanoid copepod).

**Reliability:** (4) not assignable

17-MAR-2004

(39) (993)

**Species:** other aquatic crustacea: Immature Scylla serrata (crabs)  
**Exposure period:** 24 hour(s)  
**Unit:** g/l **Analytical monitoring:** no data  
**EC50:** = 7.14 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.

Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 5.79, 4.62 and 3.67 g/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Immature Scylla serrata (crabs) were exposed in static saltwater during the summer season.

**Reliability:** (4) not assignable

17-MAR-2004

(39) (920)

**Species:** other aquatic crustacea: Immature Scylla serrata (crabs)  
**Exposure period:** 24 hour(s)  
**Unit:** g/l **Analytical monitoring:** no data  
**EC50:** = 9.11 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.

Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 8.04, 7.33 and 6.08 g/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Immature Scylla serrata (crabs) were exposed in static saltwater during the monsoon season.

**Reliability:** (4) not assignable

17-MAR-2004

(39) (920)

**Species:** other aquatic crustacea: Immature Scylla serrata (crabs)  
**Exposure period:** 24 hour(s)  
**Unit:** g/l **Analytical monitoring:** no data  
**EC50:** = 11.29 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.

Similar tests were conducted for 48, 72 and 96 hours and gave LC50 values of 10, 8.93 and 7.63 g/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Immature Scylla serrata (crabs) were exposed in static saltwater during the winter season.

**Reliability:** (4) not assignable

17-MAR-2004

(39) (920)

**Species:** other aquatic crustacea: Scylla serrata (crabs)  
**Exposure period:** 24 hour(s)  
**Unit:** g/l **Analytical monitoring:** no data  
**EC50:** = 5.61 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.

Similar tests were conducted for 48 and 72 hours and gave LC50 values of 4.41 and 3.29 g/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Scylla serrata (crabs) were exposed in static saltwater during the breeding season.

**Reliability:** (4) not assignable

17-MAR-2004

(39) (920)

**Species:** other aquatic crustacea: *Scylla serrata* (crabs)  
**Exposure period:** 24 hour(s)  
**Unit:** g/l **Analytical monitoring:** no data  
**EC50:** = 7.14 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
It is noted that the EC50-values from this study are the only unusual high values for this endpoint; with respect to the water solubility of benzene (1.8 g/l) the validity is doubtful.  
Similar tests were conducted for 48 and 72 hours and gave LC50 values of 5.79 and 4.62 g/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** *Scylla serrata* (crabs) were exposed in static saltwater during the non-breeding season.

**Reliability:** (4) not assignable  
17-MAR-2004

(39) (920)

**Species:** other: *Aedes aegypti* (Diptera)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** 12.9 -  
**EC50:** = 59 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** No information on analytical monitoring.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 25 fourth-instar larvae of *Aedes aegypti* (mosquito) (Rockefeller strain) were exposed in a static system at 24-26 degree C to various concentrations of benzene in 200 ml solution in 300 ml glass bowls (4 bowls/trial, at least 3 trials).

**Test substance:** purity: no data

**Reliability:** (4) not assignable

**Flag:** Risk Assessment

17-MAR-2004

(99)

**Species:** other: *Aedes aegypti* (Diptera)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC0:** = 170 -  
**EC50:** = 200 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten third instar *Aedes aegypti* (mosquito) larvae were exposed at 26 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982). The actual concentrations of benzene during the experiment were not measured, and the values presented are based on the quantity added at the beginning of the experiment.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (2) valid with restrictions  
17-MAR-2004 (1052)

**Species:** other: *Brachionus calyciflorus* (Rotifera)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** > 1000 -

**Method:** other  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Remark:** No information about analytical monitoring.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** *Brachionus calyciflorus* (rotifer) neonates were exposed at 25 degree C in moderately hard, synthetic freshwater.

**Reliability:** (4) not assignable  
17-MAR-2004 (1063)

**Species:** other: Brachyonus calyciflorus (Rotifera)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC50:** > 1000 -

**Method:** other  
**Year:** 1992  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In the same study, marine rotifers (Brachyonus plicatilis) were also used and the LC50 value was also >1000 mg/l. Significant methodological deficiencies  
 No analytical monitoring (possible adsorption of benzene to polystyrene culture wells), no information if culture plates were sealed to reduce evaporation of test compound.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Animals were treated with benzene (plus control) in sterile, 24-well polystyrene tissue culture plates as follows:  
 5 concentrations of benzene (plus control), added from stock solution in ethanol,  
 10 animals/ml incubate, 3 replicates/ conc.  
 water: standard synthetic freshwater (EPA), temperature: 25 degree C, pH 7.4-7.8, hardness 80-100 mg/l as CaCO<sub>3</sub>, alkalinity 60-70 mg/l,  
 The number of dead animals was counted after 24 h.

**Test substance:** Purity: 86%.  
**Reliability:** (3) invalid  
 17-MAR-2004 (354)

**Species:** other: Chironomus gr. thummi (Diptera)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 100 -

**Method:** other  
**Year:** 1993  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** At least 5 larvae of Chironomus gr. thummi were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C. The animals (age not specified) were obtained from commercial source.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (1049)

**Species:** other: Cloeon dipterum (Ephemeroptera, Insecta)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 34 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Result:** In this study, the 48 h LD50 values of benzene for several aquatic macroinvertebrates from different taxonomical groups have been determined to obtain information on the relative susceptibility of macrobenthos to aquatic toxicants.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 larvae of Cloeon dipterum (mayfly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C. The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1049)

**Species:** other: Corixa punctata (Heteroptera, Insecta)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 48 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 Corixa punctata (water boatman) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C. The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1049)

**Species:** other: Culex pipiens (Diptera)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC0:** = 40 -  
**EC50:** = 71 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten third instar Culex pipiens (mosquito) larvae were exposed at 26 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982). The actual concentrations of benzene during the experiment were not measured, and the values presented are based on the quantity added at the beginning of the experiment.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (2) valid with restrictions  
17-MAR-2004 (1052)

**Species:** other: Erpobdella octoculata (Hirudinea)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** > 320 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** At least 5 Erpobdella octoculata were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.

The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1049)

**Species:** other: Hydra olicactis (Hydrozoa)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC0:** = 24 -  
**EC50:** = 34 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Groups of ten Hydra oligactis were exposed in closed vessels at 17 degree C in 50 ml Dutch Standard Water (Canton J.H. & Slooff W. Chemosphere 9, 891-907, 1982) to various concentrations of benzene for 48 h at 19-21 degree C.

**Test substance:** The animals were obtained from laboratory cultures. Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (1049)

**Species:** other: Ischnura elegans (Odonata, Insecta)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 10 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** At least 5 larvae of Ischnura elegans (blue-tailed damselfly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C. The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (1049)

**Species:** other: Nemoura cinerea (Plecoptera, Insecta)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 130 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** At least 5 larvae of Nemoura cinerea (stonefly) were exposed in closed vessels with Dutch Standard Water (Canton J.H. & Slooff W. Ecotoxic. Envir. Saf. 6, 113-128, 1982) to various concentrations of benzene for 48 h at 19-21 degree C. The animals (age not specified) were collected from the river Rhine.

**Test substance:** purest grade commercially available (>98% pure).

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

17-MAR-2004

(1049)

**Species:** other: Paracentrotus lividus (Echinodermata)  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no  
**EC :** .078 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In the same study, sea urchin sperm cells were treated for 5-90 min with 10 uM benzene (0.78 mg/l) prior to insemination of untreated eggs and subsequent development of embryo to the pluteus larval stage.

**Results:**

No spermiotoxicity was observed.

The no. of developmental defects in larvae was increased:

Control: 15.9+/-3%, 10 uM benzene: 65.9+/-15.3%.

Significant methodological deficiencies

Only one concentration used.

The following effects of benzene were noted:

significant increase in developmental defects:

control: 11.8+/-6.7% (S.D.), 1ug/l benzene: 29.5+/-14.3%,  
 p<0.05

elevated metaphase/anaphase ratio:

control: 2.2+/-0.8, benzene: 3.5+/-0.5, p<0.001

No effect on the no. of mitoses/embryo, % of interphase embryos, % total mitotic aberrations and % anaphase aberrations was noted.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Mediterranean sea urchin (*Paracentrotus lividus*) embryos were incubated for 48 h, from the zygote to the pluteus larval stage, with 1 µm Benzene (0.078 mg/l) in natural filtered seawater (salinity 37.6-37.8 g/l, pH 8.2-8.4, temperature 20 degree C).  
No further details of the methods are reported in this study.

**Test substance:** over 99% pure (Merck, FRG).  
**Reliability:** (3) invalid  
17-MAR-2004 (865)

#### 4.3 Toxicity to Aquatic Plants e.g. Algae

**Species:** *Ankistrodesmus falcatus* (Algae)  
**Endpoint:** other  
**Exposure period:** 4 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 310 -

**Method:** other  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Result:** The EC50 was reported in mmoles/l (3.97 mmoles/ corresp. to 310 mg/l).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Algae (400000-700000 cells) were exposed in the log phase of growth, under static conditions at 20 degree C for 4 h under illumination with 5000 Lux. Test were carried in 15 ml medium in capped 25 ml Erlenmeyer flasks.  
Benzene was added from stock solution in acetone. Controls were incubated with acetone only and additional controls were incubated in the dark.  
Inhibition of uptake of <sup>14</sup>C-carbonate was taken as a measure for the toxic effect of benzene.

**Test substance:** Purity of test substance and of <sup>14</sup>C-labelled carbonate not reported.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (1274)

**Species:** Chlamydomonas sp. (Algae)  
**Endpoint:** other  
**Exposure period:** 3 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 461 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Exponential phase Chlamydomonas angulosa (green algae, 680 Indiana collection) cells ( $5 \times 10^4$  cells/ml), grown axenic in Bolds Basal Medium at a 12 h dark/12 h light cyclus, were exposed in glass stoppered flasks under static conditions at 19 degree C and pH 6.5. A dark set of controls was also run. Benzene was added from saturated stock solutions in medium. Radiolabelled carbon dioxide uptake (1.25 uCi/100 ml) from NaHCO<sub>3</sub> (purity not specified) was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.

**Test substance:** No information on the purity of the test substance was given.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
 17-MAR-2004

(545)

**Species:** Chlorella vulgaris (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 525 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Remark:** In the study, the effects of benzene on growth of the unicellular algae Chlorella vulgaris (culture no. 29, Indiana University Culture Collection of Algae) was studied.  
 Significant methodological deficiencies  
 The concentration of benzene in the culture medium at 20 degree C was calculated from reported solubility data of a saturated benzene solution in pure water at 25 degree C. Actual benzene concentrations were not measured.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** 30000-100000 cells were incubated with various concentrations of benzene in 50 ml Bolds Basal Medium at 18-22 degree C in cotton-plugged 125 ml Erlenmeyer flasks under a 12 h light/12 h dark cycle. Growth was recorded by determination of cell number.  
 Effective concentrations refer to nominal concentrations at

the beginning of the experiment.  
Results: 525 mg/l benzene led to a 50% reduction in cell number after one day. At 25 mg/l to 500 mg/l, a lag-phase in growth proportional to the initial nominal benzene concentration was observed. Subsequently, growth of benzene-treated and control cultures did not differ significantly. This effect could be related to rapid volatilisation of benzene from the cultures, but no measurement of actual concentrations was performed.

**Test substance:** Purity not specified.  
**Reliability:** (3) invalid  
17-MAR-2004 (606)

**Species:** Chlorella vulgaris (Algae)  
**Endpoint:** other  
**Exposure period:** 3 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 312.5 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Exponential phase Chlorella vulgaris (green algae, 260 Indiana collection) cells (20 x 10<sup>4</sup> cells/ml), grown axenic in Bolds Basal Medium at a 12 h dark/12 h light cyclus, were exposed in glass stoppered flasks under static conditions at 19 degree C and pH 6.5. A dark set of controls was also run. Benzene was added from saturated stock solution in medium.  
Radiolabelled carbon dioxide uptake (0.5 uCi/100 ml) from NaHCO<sub>3</sub> (purity not specified) was measured as a sign of effects on photosynthesis. This may indicate a biomass effect but no details were given.

**Test substance:** No information on the purity of the test substance was given.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
18-MAR-2004 (545)

**Species:** Dunaliella tertiolecta (Algae)  
**Endpoint:** growth rate  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC0:** > 100 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Growth was not inhibited up to 100 mg/l, the highest concentration tested. Instead, growth was stimulated to ca. 150% of control at 1 mg/l, the lowest concentration tested.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Axenic cultures of Dunaliella (Clone, Dun, WHOI) were incubated under the following conditions:  
12h dark/ 12h white light (ca. 4000 uW/cm<sup>2</sup> at 380-700 nm)  
125 ml flasks with rubber stopper, 3 flasks/concentration.  
50 ml enriched filtered seawater  
temperature: 18 degree C.  
Cell density not specified, but such that growth was not limited due to CO<sub>2</sub>-shortage.  
Benzene concentration was monitored by gas chromatography. Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.  
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

**Test substance:** Spectro-grade benzene was tested.  
**Reliability:** (2) valid with restrictions

18-MAR-2004 (313)

**Species:** Microcystis aeruginosa (Algae, blue, cyanobacteria)  
**Endpoint:** biomass  
**Exposure period:** 8 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** > 1400 -

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Remark:** Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.  
 No detectable effect on concentration and total biomass was noted.  
 No data regarding actual concentrations during exposure period presented, possible evaporation of test substance during exposure period cannot be ruled out.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** 10 ml test solution (algae in defined medium with appropriate concentration of test substance) is incubated at 27 degree C in capped Kapsenberg culture vials for 8 days under static conditions.

**Reliability:** (3) invalid  
 17-MAR-2004

(147) (149)

**Species:** Phaeodactylum tricornutum (Algae)  
**Endpoint:** other: inhibition of net photosynthesis  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**LOEC:** = 50 -

**Method:** other  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Result:** After 24 h of exposure, 50 mg/l, the lowest concentration tested, reduced net photosynthesis to about 80% of controls. At 100, 200, and 300 mg/l, rates were reduced to 68%, 26% and 3% of control, respectively.  
 After 2 h of treatment, 50 mg/l had no effect, but 100 mg/l reduced photosynthesis to 83% of control. At 200, 300, and 400 mg/l, photosynthesis was reduced to 61%, 37%, and 0% of control, respectively.

In a 96h growth inhibition test a LOEC of 50 mg/l was found.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The marine diatom algae was exposed to benzene in glass stoppered bottles as follows:  
 salinity of growth medium: ca. 30g/l  
 pH: 7.5  
 temperature: 15 degree C  
 illumination: 7W/m2  
 cell density: 2-3 million cells/ ml  
 Benzene was added as stock solution in ethanol (50 ul stock

sol./ 20 ml culture), control received ethanol.  
Oxygen exchange was measured with an oxygen electrode in a closed chamber.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (650)

**Species:** Scenedesmus quadricauda (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 7 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** > 1400 -

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Remark:** Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.  
No detectable effect on concentration and total biomass was noted.  
Unsuitable test system  
No data regarding actual concentrations during exposure period presented, possible evaporation of test substance during exposure period cannot be ruled out.  
No information whether the algae were in exponential growth during the whole exposure period.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** 10 ml test solution (algae in defined medium with appropriate concentration of test substance) is incubated at 27 degree C in capped Kapsenberg culture vials for 7 days under static conditions.

**Reliability:** (3) invalid  
17-MAR-2004 (149) (150)

**Species:** Scenedesmus sp. (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC10:** > 1360 -  
**EC50:** > 1360 -

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Unsuitable test system  
The test was performed in compliance with the test guideline of the Federal Environmental Agency, FRG. However, according to the authors, the method is not suitable for testing volatile substances such as benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Scenedesmus subspicatus (green algae) (ca. 10+4 cells/ml) were exposed to benzene under static conditions (300 ml Erlenmeyer flasks with Kapsenberg caps) in nutrient medium at 20-24 degree C for 96 h. Cells were continuously illuminated with 120 uE/m2 x sec. Cell growth was determined by photometric determination of cell density.

**Test substance:** 99.7% pure.  
**Reliability:** (3) invalid  
18-MAR-2004

(405)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 72 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** = 29 -

**Method:** OECD Guide-line 201 "Algae, Growth Inhibition Test"  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Effect value is related to mean measured concentration.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The OECD Test Guideline was modified to allow for the testing of volatile compounds (closed vessels). The concentration causing 50% growth inhibition was measured.

**Test substance:** Purity: Carlo Erba GC standards.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

18-MAR-2004

(390)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 8 day(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** = 41 -

**Method:** other  
**Year:** 1990  
**GLP:** no data  
**Test substance:** other TS

**Method:** Analysis of benzene in both liquid and headspace of incubation vials was performed by GC. Concentrations were determined in vials containing a hydrocarbon mixture with benzene over a period of 12 days. No significant changes in the concentrations were observed.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The bioassay was performed using Bristol medium with added sodium bicarbonate (0.4%, w/v) to supplement the carbon supply and allow algal growth under conditions of restricted gas exchange.

The pH was adjusted to pH 7.0 and the medium was inoculated with  $1 \times 10^5$  cells/ml in the exponential growth phase. Bioassays were performed using 4 replicate hypo-vials (125 ml size containing 50 ml solution) to at least 5 concentrations of benzene. Neat benzene was injected into the exposure vials through the valve. Cultures were shaken at 100 rpm and exposed to benzene for 8 days under a 16h light:8h dark cycle (illumination 32 uEinstein/m<sup>2</sup>xsec). At the end of exposure, the cell number in each vial was determined by direct counting using a haemocytometer. Additionally, chlorophyll content, dry weight and absorbance were also measured after 4, 6, and 8 days and compared to ascertain which parameter would be the most sensitive indicator of algal growth inhibition.

**Test substance:** Supplied by J.T. Baker, Phillipsburg, New Jersey, "photrex" grade.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

18-MAR-2004

(500)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 14 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC10:** = 155 -  
**EC50:** = 292 -  
**EC90 :** = 552 -

**Method:** other  
**Year:** 1988  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Details of the method not reported in this study, but in an earlier publication.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Algae were exposed for 14 days under static conditions as described in an earlier publication (Gaur J.P. & Kumar H.D. Environ. Pollut. Ser. A25, 77-85, 1981).

**Reliability:** (4) not assignable  
17-MAR-2004 (395)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 96 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**NOEC:** = 600 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Documentation insufficient for assessment  
Several parameters not reported (open or closed system, no confidence limits), no analytical monitoring.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Log phase cells were exposed (50000 cells/ml) in 150 ml freshwater at 26 degree C for 96 h and the effect on population growth was noted.

**Test substance:** Purity: purest grade available (at least >98% pure, no further information given).

**Reliability:** (4) not assignable  
17-MAR-2004 (1052)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** growth rate  
**Exposure period:** 72 hour(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC10:** 34 -  
**EC50:** 100 -  
**EC90 :** > 100 -

**Method:** Directive 92/69/EEC, C.3  
**Year:** 2000  
**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Measured concentrations at the beginning of the test were between 56 to 73 % of the added concentrations, and this can be attributed to evaporative losses during the spiking and during the serial dilution of test solutions. During the test benzene concentrations remained stable between 83 and 100% of the measured concentration at the start of the test. Effect values are related to measured concentrations at test start.  
The growth rate in the controls was 0.091/h, and the present test thus fulfils the validity criteria given in the guidelines (0.038/h).

**Result:** Effect values related to biomass are as follows:  
EbC10 = 8.3 mg/l  
EbC50 = 28 mg/l  
EbC90 = 94 mg/l

**Source:** German Rapporteur

**Test condition:** Test solutions of benzene were prepared by direct addition and subsequent serial dilution with algal medium. The test was carried out at reduced algal densities ( $10^3$  cells/ml), at increased addition of NaHCO<sub>3</sub> (300 mg/l instead of 50 mg/l as specified in the guideline) and at a reduced algal medium pH (pH 7) in order to allow the test to be carried out in closed flasks without headspace. Furthermore, the medium contained Fe-citrate, because the growth of the algae would be erratic in the absence of complexed iron.  
Daily algal measurements were based on electronic particle counting, and daily measurements of benzene concentrations were based on liquid-liquid extraction and subsequent GC-MS analysis. On request of the sponsor each flask was sampled only once, and was then sacrificed.  
Incubation was performed under continuous standard illumination ( $60-120 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) on an orbital shaker at  $23 \pm 2^\circ\text{C}$ .  
In the presence of algae, the pH increased with increasing algal density (pH 7.5 - 8.8).

**Test substance:** >99.5%, ACS grade  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment

17-MAR-2004

(1141)

**Species:** Selenastrum capricornutum (Algae)  
**Endpoint:** other: inhibition of photosynthesis  
**Exposure period:** 4 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC5 :** 10 -  
**EC16 :** 100 -  
**EC95 :** 1000 -

**Method:** other  
**Year:** 1979  
**GLP:** no data  
**Test substance:** other TS

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Benzene was tested at 10, 100 and 1000 mg/l.  
 Rapidly-growing culture ( $1.25 \times 10^7$  cells/ml) were added to the benzene solutions. Radiolabelled sodium hydrogen carbonate ( $^{14}\text{C}$ ) was added after 2 hours incubation at 24 degree C and after a further 2 hours, the cells were arrested by the addition of formalin. Photosynthesis was determined by measurement of fixed  $^{14}\text{C}$ .

**Test substance:** Reagent grade benzene was used.  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (408)

**Species:** Skeletonema costatum (Algae)  
**Endpoint:** biomass  
**Exposure period:** 7 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** = 200 -

**Method:** other  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Remark:** Original reference not available  
 When exposed for 8 and 12 hours, the EC50 values for photosynthesis effects were 300 and 80 mg/l respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Algae were exposed in saltwater and effects on photosynthesis noted.

**Reliability:** (4) not assignable  
 17-MAR-2004 (38) (154)

**Species:** Skeletonema costatum (Algae)  
**Endpoint:** growth rate  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** > 100 -  
**EC :** -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Growth was not inhibited up to 10 mg/l and was slightly inhibited (ca. 20% inhibition) at 100 mg/l, the highest concentration tested.  
In further experiments, 10 mg/l benzene caused a depression of growth (to 17% of control) for the first 160 h of culture, followed by a stimulation of growth afterwards. At 20 mg/l benzene, almost no growth was observed within 160 h.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Axenic cultures of the diatom Skeletonema costatum (Clone, Skelly, WHOI Culture collection) were incubated under the following conditions:  
12h dark/ 12h white light (ca. 4000 uW/cm<sup>2</sup> at 380-700 nm)  
125 ml flasks with rubber stopper, 3 flasks/concentration.  
50 ml enriched filtered seawater  
temperature: 18 degree C.  
Cell density not specified, but such that growth was not limited due to CO<sub>2</sub>-shortage.  
Benzene concentration was monitored by gas chromatography. Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.  
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

**Test substance:** Spectro-grade benzene was tested.  
**Reliability:** (2) valid with restrictions  
17-MAR-2004 (51) (313)

**Species:** other algae: Akrosiphonia sonderi (green algae)  
**Endpoint:** other  
**Exposure period:** 2 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** < 350 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Result:** Photosynthetic oxygen production was inhibited to 67%, 36 %, and 5% of control by 175 mg/l, 350 mg/l, and 525 mg/l, respectively.  
 Control samples were not affected by addition of ethanol (50 ul/20 ml medium).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Acrosiphonia sonderi (green algae) were exposed in a respiratory chamber in saltwater at 5 degree C for 2 h, and the effect on photosynthesis, which may reflect a biomass effect, was noted by determination of oxygen development by means of a Clark electrode.  
 Benzene was added as a stock solution in ethanol.

**Test substance:** Purity: Analytical grade  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (649)

**Species:** other algae: Amphidinium carterae (Dinoflagellate)  
**Endpoint:** growth rate  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC50:** > 50 -  
**EC :** = 1 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Growth was inhibited to 60% of control at both 1 mg/l, the lowest concentration tested, and at 50 mg/l.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** Axenic cultures of Amphidinium carterae (Clone, Amphi 137, WHOI) were incubated under the following conditions:  
 12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)  
 125 ml flasks with rubber stopper, 3 flasks/concentration.  
 50 ml enriched filtered seawater  
 temperature: 18 degree C.  
 Cell density not specified, but such that growth was not limited due to CO2-shortage.  
 Benzene concentration was monitored by gas chromatography. Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.  
 Growth was measured by direct cell count (hemacytometer) and

by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

**Test substance:** Spectro-grade benzene was tested.  
**Reliability:** (2) valid with restrictions  
17-MAR-2004 (313)

**Species:** other algae: Cricosphaera carterae (coccolithophorid)  
**Endpoint:** growth rate  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC50:** > 100 -  
**EC :** > 10 -

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Growth was not inhibited up to 10 mg/l. At 100 mg/l, the highest concentration tested, benzene inhibited growth to about 70% of controls.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Axenic cultures of Cricosphaera carterae (Clone, 156, Plymouth) were incubated under the following conditions:  
12h dark/ 12h white light (ca. 4000 uW/cm2 at 380-700 nm)  
125 ml flasks with rubber stopper, 3 flasks/concentration.  
50 ml enriched filtered seawater  
temperature: 18 degree C.  
Cell density not specified, but such that growth was not limited due to CO2-shortage.  
Benzene concentration was monitored by gas chromatography. Values given refer to theoretical values, not measured concentrations, which, after 3 days of culture, dropped to ca. 20-50% of the calculated initial amount.  
Growth was measured by direct cell count (hemacytometer) and by in vivo chlorophyll fluorescence on the 2nd or 3rd day of log. growth.

**Test substance:** Spectro-grade benzene was tested.  
**Reliability:** (2) valid with restrictions  
17-MAR-2004 (313)

**4.4 Toxicity to Microorganisms e.g. Bacteria**

**Type:** other: Microbial toxicity in soil medium  
**Species:** other bacteria: Polytox commercial microbial test culture  
**Exposure period:** 10 hour(s)

**Year:** 1998  
**GLP:** no

**Remark:** A laboratory procedure using automated respirometry was developed to measure microbial toxicity in soils as a function of respiration inhibition. Sterilized sandy loam soil; source: agric field, N.M USA, organic content 0.7%, autoclaved 7hr/4d, 200g/test system; remoisturized w/ dH2O to 33, 50, 80 and 100% moisture to determine % moisture effects on toxicity. Microbes: Polytox, commercial freeze-dried product - blend of 12 strains of aerobic microbes- supp Polybac Corp. Bethlehem, PA. EPA recommended microbial culture for effluent tox testing(doc 440/4-87-005, 7/1987). Cells resuspended in nutrient buffer, and 1:10 dilution of resuspended cell volume to soil mass used for testing. Cell density of inoculum not reported. Acetone was used as a cosolvent for chemical addition. Concentration verification of spiked soils by GC/MS indicated homogenous soil concentrations. During a 10 hr test duration, respiration was monitored. IC50 calculated based on reduction of oxygen uptake rate in chemical test systems versus control reator. The test protocol was reproducible with a mean standard deviation of 0.08 mg chemical/g of soil for eight chemicals. Effect of acetone (as cosolvent) is unclear, since solvent blank (0.5 ml acetone/200 g soil) preparation is described as a control system. Determination of IC50 is calculated by comparison of respiration rates of chemicals to control; it is not clear if the control contains only soil, or acetone in soil. IC50 values for acetone are also reported, which infers soil blanks where used as comparison. It is also unclear whether the acetone concentration used as co-solvent affected respiration. Toxicity is not significantly affected by % mositure for these chemicals.

**Result:** Benzene IC50 Values at Different Soil Moisture-Holding Capacities (mg chemical/g soil)

	Moisture -holding capacity					
	33%	50%			80%	100%
IC50	0.51	0.51	0.48	0.30	0.75	0.71
n1	4	6	6	7	4	4
r2	0.990	0.916	0.985	0.690	0.844	0.965

Correlation between toxicity tests done in aqueous medium and soil for Polytox test culture was statistically significant and yielded an r2 of 0.816.

In comparison, benzene was determined to have an IC50 value of 679 mg/l for the Polytox culture in aqueous medium.

**Source:** Exxon Biomedical Scineces, East Millstone, NJ, USA

Exxon Chemical Europe Inc. Bruxelles  
EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Reliability:** (4) not assignable (46)  
29-JUL-2002

**Type:** aquatic  
**Species:** Pseudomonas putida (Bacteria)  
**Exposure period:** 8 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** = 92 -

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Remark:** No detectable effect on concentration and total biomass was noted.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Reliability:** (3) invalid (151)  
17-MAR-2004

**Type:** aquatic  
**Species:** Pseudomonas putida (Bacteria)  
**Exposure period:** 16 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** = 92 -

**Method:** other: cell multiplication inhibition test  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Method:** Endpoint: toxicity threshold (TGK) for cell multiplication inhibition.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Established cultures of Pseudomonas putida were incubated with various concentrations of benzene in a total volume of 100 ml in 300 ml Erlenmeyer flasks with cotton lined plastic cups for 16 h at 25 degree C.  
The number of cells, which served as a measure to determine cell multiplication inhibition, was determined turbidimetrically.  
No analytical monitoring of the actual benzene concentrations was performed.

**Test substance:** Purity: no data  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
17-MAR-2004 (150)

**Type:** aquatic  
**Species:** Tetrahymena pyriformis (Protozoa)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** 391 -

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Result:** Value reported is the acute toxicity threshold level, i.e. the intermediate concentration between the lowest and highest concentration causing cell death and permitting replication, respectively.  
 In the study, the value is given as 5010 mmol/m3.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The ciliate *T. elliotti* (= *T. pyriformis*; strain L1630/1C) was used. Ca. 500 cells were treated with different concentrations of benzene in a 10 ml sterile, gas tight syringe to avoid volatilisation of benzene. After 24 h, cell death was recorded using cessation of ciliary movement as criterion.  
 Benzene solutions were prepared from a saturated stock solution, which concentration was determined by GC analysis.

**Test substance:** Purity: 99%  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (954)

**Species:** Entosiphon sulcatum (Protozoa)  
**Exposure period:** 72 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC0:** > 700 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Remark:** Endpoint: toxicity threshold (TGK) for cell multiplication.  
 No measurement of actual concentrations; no data about purity of test substance.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** 20 ml test solution (*Entosiphon* in defined medium with appropriate concentration of test substance) is incubated at pH 6.9 and 25 degree C in metal-cap stoppered 300 ml Erlenmeyer flasks for 72 h under static conditions.  
 The number of cells is determined by means of a cell counter.

**Reliability:** (2) valid with restrictions  
 17-MAR-2004 (148)

**Type:** other  
**Species:** activated sludge  
**Exposure period:** 15 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**IC50 :** = 520 -

**Method:** other  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The test was conducted in a sealed bottle to prevent loss by volatilization. The concentration that inhibited the bacterium culture by 50%, at 35°C, was investigated. Uninhibited controls were used. The seed bacteria were aerobic heterotrophs obtained from the mixed liquor of an activated sludge wastewater treatment plant. Tests were run with 25 ml liquid volume at a pH of 7 in an atmosphere with a N<sub>2</sub>/O<sub>2</sub> ratio of 1:1 at a temperature of 35 degree C. The test was conducted in 125 ml serum bottles sealed to prevent by volatilisation. Carbon dioxide was absorbed with NaOH-solution. Inhibition of oxygen uptake was used as criterion for toxic effects. The test procedure is not a standard assay but was designed to mimic the standard anaerobic toxicity assay (ATA). The cell concentration was typical of the AFNOR and ETAD standard assays (King E.F. & Dutka B.J. In: Toxicity Testing Using Microorganisms. Vol. 1. Chpt. 5. Edited by G. Bitton & B.J. Dutka, CRC Press, Inc., Boca Raton, 1986). The culture was shaken during exposure to allow for aerobic conditions.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 18-MAR-2004 (120)

**Type:** other  
**Species:** Anacystis aeruginosa (Bacteria)  
**Exposure period:** 4 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**EC20 :** = 50 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The effect on photosynthesis was examined at 24 degree C. No further details were available.

**Reliability:** (4) not assignable  
 17-MAR-2004 (37) (409)

**Type:** other  
**Species:** Nitrosomonas sp. (Bacteria)  
**Exposure period:** 24 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**IC50 :** = 13 -

**Method:** other  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The concentration that inhibited the bacterium culture by 50%, at 25°C, was investigated. Uninhibited controls were used.  
 The test was conducted in sealed 125 ml bottles to prevent loss by volatilization. 50 ml liquid, pH 6.5-8.0, was incubated at 25 degree C with constant shaking for 24 h under an atmosphere with a N2/O2 ration of 1.6:1. The seed bacteria were Nitrosomonas obtained from the mixed liquor of an activated sludge plant treating meat-packing, rendering and hide-curing wastewater. The inhibition of ammonia consumption was used as the criterion for toxic inhibition. Ammonia was measured using an ammonia sensitive electrode.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (120)

**Type:** other  
**Species:** other bacteria: methanogens  
**Exposure period:** 48 hour(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**IC50 :** = 1200 -

**Method:** other  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Test condition:** The test was conducted on methanogens according to the method of Owen et al., 1979. Bacteria from an enriched 10-year established laboratory culture were used. Bacteria were exposed to benzene in sealed 125 ml serum bottles to prevent loss of benzene by volatilization. The concentration that inhibited the bacterium culture by 50%, at 35 degree C, was investigated. The anaerobic toxicity assay used measures the inhibition of gas production as the criterion for toxicity.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
 17-MAR-2004 (120)

**4.5 Chronic Toxicity to Aquatic Organisms****4.5.1 Chronic Toxicity to Fish**

**Species:** Oncorhynchus mykiss (Fish, fresh water)  
**Endpoint:** other: percentage of normal larvae at hatch, percent survival  
**Exposure period:** 27 day(s)  
**Unit:** mg/l **Analytical monitoring:** yes

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS

**Result:**

conc. (mg/l)	% hatchability	% survival normal organisms at hatching	4 d post hatch
0.013	90	90	90
0.021	82	80	80
0.62	67	63	63
5.02	60	56	55

Log probit analysis was used by the authors to determine the LC50 at hatching and 4 days after hatching. Values of 8.64 resp. 8.25 mg/l were obtained.

No NOEC or EC10 was determined by the authors. Therefore an EC10-value was derived by probit analysis on the basis of the available test results. An EC10-value of 3.5 µg/l could be determined that can be regarded as NOEC for 23-27 day exposu

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The toxicity of benzene was tested in an embryo-larval test with Oncorhynchus mykiss as test organism. In a flow-through system (temperature: 13 °C; dissolved oxygen: 9.8 mg/l; water hardness: 104.3 mg/l CaCO<sub>3</sub>; pH: 8.0 ) eggs were exposed to the test substance within 30 minutes after fertilization. Four benzene concentrations between 0.013 mg/l and 5.02 mg/l were tested. Exposure was maintained through 4 days after hatching. Average hatching time for Oncorhynchus mykiss was 23 days. Benzene concentration was measured daily by GLC or HPLC. One test parameter was the egg hatchability, including all embryos (normal or aberrant). Another test parameter was the survival of normal organisms, determined at hatching and 4 days posthatching. Normal organisms were defined as those animals that were free of gross teratic defects.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

18-MAR-2004

(109)

**Species:** Pimephales promelas (Fish, fresh water)  
**Endpoint:** other: percentage of normal larvae at hatch, percent survival, growth effects.  
**Exposure period:** 32 day(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**LOEC:** 1.6 -

**Method:** other: early life stage (ELS) test  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Result:** The major test endpoints were percentage of normal larvae at hatch, percent survival and growth effects such as wet weight, length and dry weight. For benzene a LOEC of 1.6 mg/l was found for the endpoints wet weight and total length. From this value a NOEC of 0.8 mg/l can be derived according to the TGD, as the LOEC was in the range of 10-20 % effect.

**Source:** German Rapporteur

**Test condition:** Larvae (max. 24 h old) were exposed to 5 benzene concentrations in the range of 1.5 to 25 mg/l (nominal) and a control in a flow-through system for 32 days (temperature: 25.5 °C, dissolved oxygen: 6 mg/l, water hardness: 46 mg/l CaCO<sub>3</sub>, pH: 7.7). 30-50 embryos per glass jars ( 7 cm diameter, 7.5 cm height), 2 glass jars per exposure tank. Hatching of the larvae was finished after 120 hours. Upon hatch 15-30 fry per test concentration were further exposed. Feeding of the fry with live brine shrimp nauplii. Water temperature, pH, DO, total hardness and total alkalinity were measured routinely for each exposure. The test concentration was measured at least twice a week during the exposure period.

**Test substance:** benzene, pesticide grade  
**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

18-MAR-2004 (982)

**Species:** Morone saxatilis (Fish, estuary, marine)  
**Endpoint:** weight of young fish  
**Exposure period:** 28 day(s)  
**Unit:** mg/l **Analytical monitoring:** no data  
**NOEC:** = 3.1 -  
**EC :** = 5.3 -  
  
**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data  
  
**Remark:** Original reference not available  
**Result:** Decrease in wet and dry weight of exposed fish at a benzene concentration of 5.3 mg/l.  
 No effect was seen on the caloric content (ash-free dry weight) of the exposed fish.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur  
**Test condition:** Juvenile fish were exposed in a saltwater, flow-through, laboratory system at 15.2-16.4 degree C. The water contained 7.5-7.9 mg/l dissolved oxygen and <0.5 mg/l ammonia. The salinity was 25-26 g/l and it had a pH of 7.7-7.8.  
**Reliability:** (4) not assignable  
 17-MAR-2004 (38) (632)

#### 4.5.2 Chronic Toxicity to Aquatic Invertebrates

**Species:** other aquatic crustacea: Ceriodaphnia dubia  
**Endpoint:** other: Survival, behavior and reproduction  
**Exposure period:** 7 day(s)  
**Unit:** mg/l **Analytical monitoring:** yes  
**NOEC:** = 3 -  
**LOEC:** = 8.9 -  
  
**Method:** other  
**Year:** 1998  
**GLP:** no data  
**Test substance:** other TS: Benzene  
  
**Result:** For benzene, the NOEL, LOEL and IC50 values in Ceriodaphnia dubia were determined to be 3.0 mg/L (38 µM), 8.9 mg/L (114 µM) and 11.6 mg/L (149 µM), respectively, in this 7-day reproductive toxicity study.  
 Toxicological effect levels were based on steady state measured concentrations of the chemicals.  
  
 For 48h exposure a LC50 of 17.2 mg/l (221 µM) was determined.  
**Source:** EXXON Biomedical Sciences East Millstone, NJ  
 German Rapporteur  
**Test condition:** Tests were conducted in artificial moderately hard water (pH: 7.6, conductivity: 208, hardness: 64, alkalinity: 88).  
 The test was performed in tightly capped glass reagent bottles with minimal headspace. Solutions were renewed daily. DO was monitored on 24 hours old solutions and remained > 7 ppm.

Survival, behaviour and reproduction were observed at the time of daily renewal.  
Concentration of benzene was measured by HPLC.

**Test substance:** purity 99.9 %  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
18-MAR-2004 (825)

**Species:** other aquatic crustacea: Callinectes sapidus  
**Endpoint:** mortality  
**Unit:** mg/l **Analytical monitoring:** yes  
**EC :** 1 -

**Method:** other  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Result:** A significantly greater number of benzene treated animals died before molting (55.8% vs. 23%).  
The mean time required to molt was significantly increased for animals exposed to benzene (control: 33 days, treated: 50 days).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Juvenile *C. sapidus* (blue crab, carapace widths 1-8 cm), collected from the wild, were kept in 750 ml artificial sea water (osmolality 280-380 osmol, temperature 23 degree C, 12h:12h dark/light cycle) for at least 2 weeks before the experiment was started. Crabs were induced to autotomize the 3rd appendage. One group was exposed to 1 mg/l benzene (nominal concentration, see below) (added as stock solution in ethanol, water with benzene was changed daily), the other received ethanol only (water was changed twice a week). To assess the concentration of benzene in the water, the actual concentrations were monitored in a control experiment by GC analysis. Concentrations fell linearly within 7 h to about 20% of initial level. It was concluded that the animals were exposed to 0.26-0.3 mg/l benzene for 18 h a day.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
17-MAR-2004 (173)

**Species:** other aquatic crustacea: Cancer magister

**Endpoint:** other

**Exposure period:** 20 day(s)

**Unit:** mg/l

**Analytical monitoring:** yes

**NOEC:** ca. .17 -

**LOEC:** ca. 1.1 -

**Method:** other

**Year:** 1977

**GLP:** no data

**Test substance:** no data

**Method:** The concentration of benzene in the test solutions was measured by UV-spectrophotometry (at 255 nm).

**Result:** Benzene had little effect on the duration of the larval stages and had no effect on the size of surviving larvae. At the lowest concentration used, benzene had no effect on survival. At the other two concentrations used, benzene led to an accelerated mortality rate compared to untreated controls. After 10 days of exposure to the highest concentration of benzene (6.5-7 mg/l), most larvae died. With 1.1-1.2 mg/l, mortality rate was less but most larvae died before day 20 of exposure.

Control mortality increased steadily during the two tests. At the end of the experiments control survival was only 10 % in the first assay and about 35 % in the second. From the poor control survival it can be concluded, that the test animals were in poor health and/or stressed by test factors other than the benzene exposure. Therefore, the result of this study cannot be used for risk assessment purposes.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Larval stages of the Dungeness crab (Cancer magister), were continuously exposed after hatching in flowing water laboratory culture systems to benzene for up to 60 days as follows:

Filtered sterile seawater, saturated with air, salinity: 29-34 g/l, temperature: 10.5-14.2 degree C, photoperiod: 11h dark/13h light, 3 benzene concentrations (0.17-0.18 mg/l, 1.1-1.2 mg/l, and 6.5-7.0 mg/l) and control.

In each test, 25 larvae/culture container were used, a total of 300 in controls and 100 in each exposure treatment.

Larvae were fed brine shrimp during exposure and were transferred to clean culture containers three times a week. Effects on survival, duration of larval development and size were employed as indicators of toxic effects.

**Reliability:** (3) invalid

**Flag:** Risk Assessment

17-MAR-2004

(172)

**Species:** Daphnia magna (Crustacea)  
**Endpoint:** other  
**Unit:** mg/l **Analytical monitoring:** no data  
**NOEC:** = 98 -  
**Method:** other  
**GLP:** no data  
**Test substance:** no data  
**Remark:** documentation insufficient for assessment  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Test condition:** Daphnia magna were exposed for partial lifetime or lifetime.  
No further details were not available in the paper.  
**Reliability:** (4) not assignable  
17-MAR-2004 (162) (1191)

## TERRESTRIAL ORGANISMS

### 4.6.1 Toxicity to Sediment Dwelling Organisms

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### 4.6.2 Toxicity to Terrestrial Plants

**Species:** Avena sativa (Monocotyledon)  
**Endpoint:** growth  
**Expos. period:** 14 day(s)  
**Method:** OECD Guide-line 208 "Terrestrial Plants, Growth Test"  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data  
**Remark:** Test system not suitable for volatile substances.  
**Result:** Up to the highest tested concentration of 1000 mg/kg no phytotoxic effects and no growth inhibition was found.  
**Source:** German Rapporteur  
**Test condition:** Soil: organic carbon content: 1.4 %; 35 % < 20 µm; pH: 6.2.  
For each test concentration 1000 g of oven-dried soil was mixed with the test substance in quartz sand.  
Seedlings of Avena sativa were exposed to 4 test concentrations (1, 10, 100 and 1000 mg/kg soil) and 1 control.  
Temperature: 18 - 20 °C, air humidity: 70 - 80 %; illumination with > 8000 lux.  
The plant vessels were controlled and weighed daily and soil humidity was adjusted.  
**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
18-MAR-2004 (637) (651)

**Species:** Brassica rapa (Dicotyledon)  
**Endpoint:** growth  
**Expos. period:** 14 day(s)

**Method:** OECD Guide-line 208 "Terrestrial Plants, Growth Test"  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Remark:** Test system not suitable for volatile substances.  
**Result:** Up to the highest tested concentration of 1000 mg/kg no phytotoxic effects and no growth inhibition was found.  
**Source:** German Rapporteur  
**Test condition:** Soil: organic carbon content: 1.4 %; 35 % < 20 µm; pH: 6.2.  
For each test concentration 1000 g of oven-dried soil was mixed with the test substance in quartz sand.  
Seedlings of Brassica rapa were exposed to 4 test concentrations (1, 10, 100 and 1000 mg/kg soil) and 1 control.  
  
Temperature: 18 - 20 °C, air humidity: 70 - 80 %; illumination with > 8000 lux.  
The plant vessels were controlled and weighed daily and soil humidity was adjusted.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
18-MAR-2004 (637) (651)

**Species:** other terrestrial plant  
**Endpoint:** growth  
**Unit:** g/l  
**EC :** .02 - .2

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Remark:** Low concentrations of benzene in the water used for watering, stimulate plant growth and root formation. In contrast, higher concentrations of 0.9-1.3 g/l (near the saturated solution of 1.8 g/l) have a growth inhibiting effect. No more details were available.  
Original reference not available  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Reliability:** (4) not assignable  
18-MAR-2004 (162) (776)

**Species:** other terrestrial plant  
**Endpoint:** growth  
**Unit:** mg/l  
**LC :** > .05 -

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** no data

**Remark:** High concentrations of benzene in air have a lethal effect on plants. No more details were available.

Original reference not available  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
 German Rapporteur

**Reliability:** (4) not assignable  
 18-MAR-2004

(162) (776)

**Species:** other terrestrial plant: barley, carrot  
**Endpoint:** other: injury of leaves

**Method:** other: flow-through  
**Year:** 1954  
**GLP:** no  
**Test substance:** no data

**Method:** Barley and carrot plants grown in flat cans were exposed to benzene vapours in the gas chamber for 30 minutes, 1 hour and 2 hours.

Concentration and exposure time were varied in a four-stage process so as to establish the minimum benzene concentration giving 90 - 100 % injury within 1 hour exposure.

The test concentrations employed for benzene were:

1.2 mmol/l = 94 g/m<sup>3</sup> (barley only)

2.7 mmol/l = 211 g/m<sup>3</sup> (barley and carrot)

3.0 mmol/l = 234 g/m<sup>3</sup> (carrot only)

The kind and extent of injury were recorded 1 day, 2 and 4 weeks after treatment.

**Result:** Percent injury produced by benzene after defined periods of time

Barley treated with benzene vapors (94 g/m<sup>3</sup>):

	for 2 h	4 h	8 h
after 1 day	0	0	trace
after 2 weeks	0	0	trace
after 4 weeks	0	0	trace

Barley treated with benzene vapors (211 g/m<sup>3</sup>):

	for 1/2 h	1 h	2 h
after 1 day	85	90	95
after 2 weeks	95	100	100
after 4 weeks	95	100	100

Carrots treated with benzene vapors (211 g/m<sup>3</sup>):

	for 1/2 h	1 h	2 h
after 1 day	0	0	0
after 2 weeks>	0	0	0
after 4 weeks	0	0	0

Carrots treated with benzene vapors (234 g/m<sup>3</sup>):

	for 1/2 h	1 h	2 h
after 1 day	10	10	10

The concentrations causing 90 - 100 % injury in a 1-hour treatment (observed 24 hours after treatment):  
Barley: 203 g/m<sup>3</sup>,  
Carrot: 305 g/m<sup>3</sup>

Concentrations used in this experiment do not have any relevance for environmental considerations.

**Source:**  
**Test condition:**

German Rapporteur  
The gas chamber used in this study was not described in further detail; there was only a reference to the paper "Currier, H.B., Hilgardia 20, 383 - 406 (1951)".  
Barley was treated 2 weeks after planting (plants in the two-leaf stage), carrots were treated 63 - 95 days after seed.

**Reliability:**  
**Flag:**  
18-MAR-2004

(2) valid with restrictions  
Risk Assessment

(269)

**Species:**  
**Endpoint:**

other terrestrial plant: tomato, barley, carrot  
other: injury of leaves

**Method:**  
**Year:**  
**GLP:**  
**Test substance:**

other: flow-through and static  
1950  
no  
other TS: Chemically pure

**Method:**

Air was passed through a vaporisation chamber containing benzene. The temperature of benzene was changed as a means of changing the benzene concentration in air.  
Tomato, barley and carrot plants grown in flat cans were exposed to benzene vapours in the gas chamber for 15 and 30 minutes, 1 hour and 2 hours, barley also 4 hours.  
The kind and extent of injury were recorded immediately after treatment and at regular intervals thereafter.  
Photographs were taken 24 hours after removal from the gas chamber.

The test concentrations employed for benzene were:  
0.00022 mol/ = 17.2 g/m<sup>3</sup> (barley only)  
0.00032 mol/l = 25.0 g/m<sup>3</sup> (all 3 species tested)  
0.00064 mol/l = 50.0 g/m<sup>3</sup> (barley only)

Other plants were sprayed with benzene in a series of a control can, 2 ml, 5 ml, 10 ml and 20 ml. 57 days after spraying the fresh weights and heights of the tops were determined and used to estimate relative growth rates.

**Result:**

Percent injury produced by benzene after defined periods of time

Barley treated with benzene vapors (17.2 g/m<sup>3</sup>):

	for 15 min	30 min	1 h	2 h	4 h
after 1 week	2	25	25	25	25
after 2 weeks	2	30	25	25	25
after 4 weeks	0	0	0	0	0

Barley treated with benzene vapors (25.0 g/m<sup>3</sup>)

	for 15 min	30 min	1 h	2 h
after 24 hours	60	85	98	98
after 1 week	60	85	98	100
after 2 weeks	50	75	98	100
after 4 weeks	30	50	100	100

Barley treated with benzene vapors (50.0 g/m<sup>3</sup>)

	for 15 min	30 min		
after 24 hours	40	100		
after 1 week	--	100		
after 2 weeks	40	100		
after 4 weeks	25	100		

Tomato treated with benzene vapors (25.0 g/m<sup>3</sup>)

	for 15 min	30 min	1 h	2 h
after 24 hours	80	90	95	100
after 1 week	75	90	98	100
after 2 weeks	60	80	95	100
after 4 weeks	40	60	90	100

Carrot treated with benzene vapors (25.0 g/m<sup>3</sup>)

	for 15 min	30 min	1 h	2 h
after 24 hours	0	85	90	95
after 1 week	0	75	85	95
after 2 weeks	0	75	85	95
after 4 weeks	0	50	80	90

Plants treated by spraying with benzene; injuries:

Barley treated with

	2 ml	5 ml	10 ml	20 ml
after 24 hours	25	50	40	80
after 1 week	25	60	40	80
after 2 weeks	5	25	25	70
after 4 weeks	0	5	5	70

Tomato treated with

	2 ml	5 ml	10 ml	20 ml
after 24 hours	50	85	90	95
after 1 week	50	85	95	95
after 2 weeks	50	90	98	100
after 4 weeks	25	85	98	100

Carrot treated with

	2 ml	5 ml	10 ml	20 ml
after 24 hours	25	40	80	95
after 1 week	25	25	75	95
after 2 weeks	10	15	50	95
after 4 weeks	20	15	40	90

Concentrations used in this experiment do not have any relevance for environmental considerations.

**Source:** German Rapporteur

**Test condition:** 25°C, air flow: 11.5 l/min; concentration of benzene in air was calculated from the amount evaporated per unit time and the air flow.

The number of plants per flat can exposed to benzene vapours and the time between planting and exposition in days were:

Tomatoes: 3 per can, 32 days after planting.

Barley: 12 per can, 14 days after planting.  
Carrots: 20 per can, 32 days after planting.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment  
18-MAR-2004 (268)

**Species:** other terrestrial plant: tobacco  
**Endpoint:** other: injury of leaves and death of plant

**Method:** other: static  
**Year:** 1938  
**GLP:** no  
**Test substance:** other TS: 90 % purity

**Method:**

1. Tobacco seedlings potted in soil in exposition chambers were exposed to specific amounts of benzene introduced into the chamber onto a filter paper. The air was mixed by a ventilator.
2. Tobacco seedlings were suspended in glass flasks with their roots covered by moist cotton encased in thin foil. After introducing the desired amount of benzene into the flask onto a bit of cotton the flask was sealed. In both cases, the volume percentage of benzene added amounted to 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 as well as a control (no benzene added). Each experiment was run in several replicates.
3. After the preliminary experiments in the static chambers, tobacco seedlings were exposed to benzene vapours in 15 l chambers with permanent gas flow (2.5 l/h for 10 hours after initial rapid blowing in the benzene vapour). All surfaces exposed to benzene vapour were protected against benzene absorption. The plants were examined 1 - 2 hours after the end of exposure. If the leaves regained their turgidity, they were regarded as uninjured. If the leaves did not regain their turgidity, and necrosis began to develop, the benzene concentration was regarded as toxic.

**Result:** The experiments in the flow-through chambers gave the following results:

Benzene concentration (Vol %)	Effect on tobacco seedlings
Tests at 24.7 - 26.1°C	
0.6	no effect
1.9 (analytical)	wilted on removal, recovery
2.4 - 2.6 (analytical)	severe wilting, recovery
2.8 (analytical)	moderate injury, necrotic areas
4.1 (analytical)	plants were killed
Tests at 8.1 - 10.0°C	
0.1 - 1.0 (calculated)	no effect
1.5 (calculated)	very slight injury, necrotic areas
2.3 (calculated)	serious injury
3.1 (calculated)	plants were killed

**Source:** German Rapporteur  
**Test condition:** The exposure to benzene vapour took place at 23.6 - 26.1°C for 12 to 19 hours in the static experiments and at 21.1 -

26.6°C or 8.1 - 10.0°C in the flow-through experiments.

The benzene concentrations in Vol % / g/m<sup>3</sup> are as follows:  
0.1 / 3.2, 0.6 / 19.2, 1.0 / 32, 1.5 / 48, 1.9 / 61, 2.3 /  
73, 2.4 / 77, 2.6 / 83, 2.8 / 89, 3.1 / 99, 4.1 / 131.

**Test substance:**

90 % purity, sulfur 0.14 %, toluene 4.0 %.

**Reliability:**

(2) valid with restrictions

**Flag:**

Risk Assessment

18-MAR-2004

(897)

**Species:**

other terrestrial plant: barley and carrot

**Endpoint:**

other: plasmolysis

**Method:**

other: static

**Year:**

1952

**GLP:**

no

**Test substance:**

no data

**Method:**

An aqueous 0.05 % solution of 2,3,5-triphenyltetrazolium chloride was applied to tap water (pH 7.8) at 25°C. After 16 to 24 hours in the dark leaf and root segments of *Elodea canadensis* showed a reduction of the salt to a red colour with toxicity low enough to permit cells to remain alive after the exposure.

For the testing of benzene toxicity, saturated solutions were prepared by agitating tap water with excess benzene. Dilutions were made by adding tap water and calculating concentrations from the saturation concentration.

Shoot segments of *Elodea canadensis* were treated with an aqueous benzene solution for 1 hour at 25°C in light (200 foot-candles = 2150 Lux) and then placed in 0.05 % 2,3,5-triphenyltetrazolium chloride solution. After 24 hours in the dark, living shoots were red and dead shoots remained green.

Test of this kind were also done with leaves and roots of barley and carrots. The exposure to aqueous benzene solutions was 8 hours, while the other parameters were the same as with the *Elodea canadensis* assays.

**Result:**

Minimum benzene concentrations lethal to barley and carrot at 25°C after 8 hours of exposure:

Barley leaf: 8.3 mmol/l = 0.65 g/l;  
barley root: 11.9 mmol/l = 0.93 g/l.

Carrot leaf: 14.2 mmol/l = 1.11 g/l;  
carrot root: 21.3 mmol/l = 1.66 g/l.

Concentrations used in this experiment do not have any relevance for environmental considerations.

**Source:**

German Rapporteur

**Test condition:**

The assays were done in capped Mason jars on a rocking-type agitator.

18-MAR-2004

(269)

**4.6.3 Toxicity to Soil Dwelling Organisms**

**Type:** artificial soil  
**Species:** Eisenia fetida (Worm (Annelida), soil dwelling)  
**Endpoint:** mortality  
**Exposure period:** 28 day(s)  
**Unit:** mg/kg soil dw  
**LC50:** > 1000 -

**Method:** OECD Guide-line 207 "Earthworm, Acute Toxicity Test"  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Method:** Earth worms (3-6 months old) were exposed to benzene in artificial soil (0.1 - 1000 mg/kg dw; dw = dry weight of substrate) for 4 weeks and monitored for any kind of effects (sublethal and lethal).

**Remark:** Test system not suitable for volatile substances.

**Result:** Benzene up to 1000 mg/kg dw did not cause any effects upon the earthworms.

**Source:** German Rapporteur

**Test condition:** Artificial soil was made by mixing white peat, bentonite, excrement of cattle, calcium carbonate, and quartz sand in quantities of 1000 g in a stainless steel pot for 10-12 minutes under simultaneous addition of 350 ml of water. Benzene (1000 mg) was mixed with 10 g of quartz sand. This sand was mixed with the above-mentioned substrate under addition of 200 ml of water. Amounts of benzene of 100 mg or less were mixed with the above-mentioned substrate as a solution in 200 ml of water.

The earth worms employed in the tests were acclimated to the artificial soil without benzene for 3-5 days.

**Reliability:** (3) invalid  
**Flag:** Risk Assessment  
 18-MAR-2004 (637) (651)

**Type:** filter paper  
**Species:** Eisenia fetida (Worm (Annelida), soil dwelling)  
**Endpoint:** mortality  
**Exposure period:** 48 hour(s)  
**Unit:** mg/cm<sup>2</sup> filter paper  
**LC50:** .1 - 1

**Method:** Directive 87/302/EEC, part C, p. 95 "Toxicity for earthworms: Artificial soil test"  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Documentation insufficient for assessment  
 According to the authors, experiments were based on the protocol of the EC-directive. However, no EC50 value but only a range is given in this study. Several experimental details were not reported (temperature, nominal or actual benzene concentrations used, purity of test compound,

concentration dependent effects on mortality rates).

**Result:** Benzene was classified as "moderately toxic" as its LC50 value fell within the the limits of this predefined toxicity class.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** The test protocol used was based on that proposed by the EEC (Edwards C.A. Personal communications relative to his coordination of developing the methodology and protocol for an earthworm testing procedure as directed by the European Economic Commission. Rothamsted Experimental Station, Harpenden, Herts, England, 1981). Benzene was added directly to moist filter paper. Groups of ten worms were exposed at each test concentration in capped glass vials. Death was recorded if the worm did not respond to gentle agitation of its anterior end. Severely morbid worms having lost its posterior end when considered alive when still responding to agitation.

**Test substance:** Technical- or analytical-grade benzene was tested (no details reported).

**Reliability:** (4) not assignable

**Flag:** Risk Assessment  
18-MAR-2004 (950)

**Type:** filter paper

**Species:** Eisenia fetida (Worm (Annelida), soil dwelling)

**Endpoint:** mortality

**Exposure period:** 48 hour(s)

**Unit:** mg/cm<sup>2</sup> filter paper

**LC50:** = .098 -

**Method:** other

**Year:** 1986

**GLP:** no data

**Test substance:** no data

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Filter paper was impregnated with benzene and the earthworms were allowed to absorb this into their bodies for an unspecified period.

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment  
18-MAR-2004 (820) (827)

**4.6.4 Toxicity to other Non-Mamm. Terrestrial Species**

**Species:** other: Xenopus laevis (Clawed toad)  
**Endpoint:** mortality  
**Expos. period:** 48 hour(s)  
**Unit:** other: mg/l  
**LC0:** = 105 -  
**LC50:** = 190 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Documentation insufficient for assessment  
Several details not reported (oxygen concentration during test, no monitoring of concentrations).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Groups of ten, 3-4 week old Xenopus laevis (clawed toad) larvae were exposed in 1 l Dutch Standard water in covered, all-glass aquaria at 19-21 degree C.

**Test substance:** Analytical grade benzene was tested.

**Reliability:** (4) not assignable

18-MAR-2004

(284) (1047) (1051)

**Species:** other: Ambystoma mexicanum (Axolotl)  
**Endpoint:** mortality  
**Expos. period:** 48 hour(s)  
**Unit:** other: mg/l  
**LC0:** = 120 -  
**LC50:** = 370 -

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Remark:** Documentation insufficient for assessment  
Several details not reported (use of solvent vehicle?, purity of test compound, no monitoring of benzene concentrations, oxygen concentration during treatment)

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test condition:** Ambystoma mexicanum (Mexican axolotl; Salamander) larvae (3-4 weeks old) were exposed in groups of ten in static freshwater (1 l of Dutch Standard water in covered glass basins) at 19-21 degree C. No further experimental details were reported in this study.

**Reliability:** (4) not assignable

18-MAR-2004

(1046) (1050)

**Species:** other: Rana pipiens (Leopard frog)  
**Endpoint:** other  
**Expos. period:** 9 day(s)  
**Unit:** ppm

**Method:** other: flow-through system  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS: reagent grade

**Method:** Eggs were exposed to 5 resp. 6 different benzene concentrations within 30 minutes of fertilization. Exposure was maintained through 4 days after hatching. Average hatching time was 5 days. Benzene concentration was measured daily by GLC or HPLC. Test parameters were egg hatchability, including all embryos (normal or aberrant) and survival of normal organisms determined at hatching and 4 days posthatching. Normal organisms were determined as those organisms that were free of gross teratic defects.

Benzene conc. [mg/l]	Percent hatchability		Percent survival of normal organisms	
	at hatching	4 days post hatch	at hatching	4 days post hatch
0.016	95	95	95	
0.048	90	90	90	
0.61	81	81	79	
2.99	64	64	62	
5.07	39		32	32

Log probit analysis was used by the authors to determine the LC50 at hatching and 4 days after hatching. For Rana pipiens values of 4.03 resp. 3.66 mg/l were calculated. Additionally, the authors determined with the same statistical method LC1- and LC10-values at 4 days posthatching. For Rana pipiens a LC1-value of 3.2 µg/l and a LC10-value of 75.6 µg/l was determined.

**Source:** German Rapporteur  
**Test condition:** Flow-through system:  
 temperature: 20.2 ± 0.5 °C;  
 dissolved oxygen: 7.5 mg/l;  
 water hardness: 96.6 ± 1 mg/l CaCO<sub>3</sub>;  
 pH: 7.7 ± 0.02.

**Reliability:** (2) valid with restrictions  
**Flag:** Risk Assessment

18-MAR-2004

(109)

**Species:** other: Ambystoma gracile (Northwestern Salamander)  
**Endpoint:** other  
**Expos. period:** 9 day(s)  
**Unit:** ppm

**Method:** other: flow-through system  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS: reagent grade

**Method:** Eggs were exposed to 5 resp. 6 different benzene concentrations within 2-8 hours postspawning. Exposure was maintained through 4 days after hatching. Average hatching time was 5.5 days. Benzene concentration was measured daily by GLC or HPLC. Test parameters were egg hatchability, including all embryos (normal or aberrant) and survival of normal organisms determined at hatching and 4 days posthatching. Normal organisms were determined as those organisms that were free of gross teratic defects.

Benzene conc. [mg/l]	Percent hatchability		Percent survival of normal organisms	
	at hatching	4 days posthatching	at hatching	4 days posthatching
0.016	99	99	99	99
0.044	97	97	97	97
0.61	89	89	89	87
2.69	75	75	75	70
5.43	49	44	44	44
36.7	31	23	23	15

Log probit analysis was used by the authors to determine the LC50 at hatching and 4 days after hatching. For Ambystoma gracile values of 6.68 and 5.21 mg/l were calculated. Additionally, the authors determined with the same statistical method LC1- and LC10-values at 4 days posthatching. For Ambystoma gracile a LC1-value of 68.2 µg/l and a LC10-value of 478.1 µg/l was determined.

**Source:** German Rapporteur  
**Test condition:** Flow-through system:  
 temperature: 20.2 ± 0.5 °C;  
 dissolved oxygen: 7.5 mg/l;  
 water hardness: 96.6 ± 1 mg/l CaCO<sub>3</sub>;  
 pH: 7.7 ± 0.02.  
**Flag:** Risk Assessment

18-MAR-2004

(109)

#### **4.7 Biological Effects Monitoring**

-

#### **4.8 Biotransformation and Kinetics**

- Type:** animal
- Method:** Oysters and clams were collected from Lake Pontchartrain, Louisiana, USA in May and June 1980 and analysed for the presence of benzene.
- Result:** One sample of oysters (*Crassostrea virginica*) was found to contain 220 µg/kg wet weight and one sample of clams (*Rangia cuneata*) contained 260 µg/kg wet weight. A second sample of clams taken from the same area had no detectable benzene (detection limits not stated).
- Source:** BP Chemicals Ltd LONDON  
German Rapporteur
- 29-JUL-2002 (355)
- Type:** animal
- Method:** The effects of benzene on various mixed-function oxydases and on cytochrome P450 was investigated.
- Remark:** Significant methodological deficiencies  
No controls treated with vehicle (corn oil) only were reported to be used.
- Result:** Cytochrome P450 content was decreased after benzene treatment to ca. 78% of control. Among the monooxygenase dependent enzymes studied, the activity of the 7-ethoxy resorufin-O-deethylase (EROD) was decreased (73% of control). The activity of the aniline 4-hydroxylase and ethylmorphine N-demethylase was not affected.
- Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur
- Test condition:** Gilthead seabream (*Sparus aurata*. weight 225-275 g) were kept in aerated, filtered seawater at 16.5-17.5 degree C and fed daily. Benzene was administered once daily for 4 days by s.c injection (0.3 ml of benzene/corn oil 1:1). Microsomes were prepared from the liver and the cytochrome P450 content and the activity of several monooxygenases were determined by standard methods.  
Controls receiving corn oil are not reported.
- Reliability:** (3) invalid
- 18-MAR-2004 (41)
- Type:** aquatic
- Method:** Between November 1980 and August 1981, quarterly, five replicate, one week composites of the final effluent from the Los Angeles County wastewater treatment plant were collected for analysis of extractable organics (EOs) and two grab samples of surficial sediments were collected for analysis of volatile organics (VOs). The sampling site was 6 km NW of the discharge zone at Pulos Verdes and a modified Van Veen sampling device was used. During June 1981, biological samples were collected at this same site. These included *Citharichthys xanthostigma* (Pacific sanddab), *Microstomus pacificus* (Dover sole), *Scorpaena guttata* (scorpionfish), *Genyonemus lineatus* (white croaker), *Sicyonia ingentis* (ridgeback prawn) and a sample of small

invertebrates collected from just above the bottom sediments (containing 74% (by weight) mysids and 23% decapod shrimp). All EOs and VOs were separated from the samples using the standard EPA protocol for analysis of priority pollutants (EPA. Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants. Environmental Monitoring and Support Laboratory, US Environmental Protection Agency, Cincinnati, Ohio, 1977) or by using previously reported methods (Young D.R. et al. J. Wat. Pollut. Control Fed. 48, 1919-1928, 1976). VOs from sediments and tissues were separated using EPA protocol method 601. Analysis was done using gas chromatography/mass spectrometry.

**Result:** Benzene was detected at a level of 220 µg/l of effluent and <1 µg/kg dry sediment. In the biological samples, benzene was present at concentrations of <1, 52, 16, 15 (liver samples), <1 (muscle sample) and 8 (whole invertebrate) µg/kg wet weight respectively.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

29-JUL-2002

(431)

**Type:** plant

**Method:** Two species of macroalgae, *Ulva lactuca* and *Hypnea musciformis* (seaweeds) were grown in tanks using circulating seawater. The algae samples were analysed by a modified headspace technique (Whelan J.K. et al. *Geochim. Cosmochim. Acta* 44, 1767-1785, 1980) using gas chromatography and mass spectrometry. Degassed distilled water and the tank water in which the algae were grown were used as blanks.

**Result:** Benzene was not detected in either of the blanks but was present in the algae at levels of 20 ng/g dry weight of algae.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

29-JUL-2002

(1249)

**Type:** plant

**Method:**

1. Vapours of benzene with radiolabeling (14-C) were applied to sterile spinach seedlings in a hermetic chamber containing the phyllosphere (i. e. the stem with the leaves). Carbon dioxide was absorbed by aqueous potassium hydroxide solution, the metabolites of benzene were analysed after the end of the exposure autoradiographically (no details on the identification of the metabolites available, only a reference to an earlier paper). For investigating the distribution of radioactivity between different cellular organelles, the leaves were homogenised and centrifuged and filtered several times in order to separate the different organelles into several fractions.
2. A chloroplast suspension prepared from 2 g leaves in a phosphate buffer was mixed with 14-C-benzene and different inhibitors (8-hydroxyquinoline, *a,a'*-bipyridyl, sodium diethyldithiocarbamate at 1 mM) and exposed to light for 3 hours. Afterwards, the chloroplasts were separated, and the

**Result:**

solution was extracted with petroleum ether. The extracts in the petroleum ether were transferred into another solvent mixture and analysed.

3. Enzyme isolates from fresh leaves in phosphate buffer were incubated with <sup>14</sup>C labeled benzene and different additives (cofactors NADH and NADPH, inhibitors 8-hydroxyquinoline, a,a'-bipyridyl, sodium diethyldithiocarbamate, each at 1 mM). After 7 hours of exposure at 22-23°C in light, the solution was extracted with petroleum ether. The extracts in the petroleum ether were transferred into another solvent mixture and analysed.

1. The radioactivity recovered from the spinach leaves was found in organic acids (84 %) and amino acids (16 %).

Distribution of <sup>14</sup>C in the organic acids:

muconic acid 37.2 %, fumaric acid 24.4 %, succinic acid 12.5 %, malic acid 9.6 %, oxalic acid 9.1 %, unidentified 7.2 %.

Distribution of <sup>14</sup>C in the amino acids: tyrosine 33.8 %, phenylalanine 25.8 %, glycine 16.2 %, aspartic acid 11.3 %, two unidentified compounds 7.4 % and 5.5 %.

The distribution between different organelles in the spinach leaves was: nuclei and cell walls 6.0 %, chloroplasts 30.7 %, mitochondria 12.5 %, microsomes 3.3 %, cytosol 47.5 %.

2. The effects on the transformation of benzene by chloroplasts in the dark and in the light were as follows (total radioactivity of nonvolatile metabolites in terms of counts per minute per g of fresh weight):

no additives: 5,300 (dark), 9,400 (light);

8-hydroxyquinoline: > 100 (dark), 500 (light);

a,a'-bipyridyl: 4,700 (dark), 7,800 (light);

sodium diethyldithiocarbamate: > 100 (dark), 800 (light).

3. The effects on the transformation of benzene by enzyme preparations were as follows (total radioactivity of nonvolatile metabolites in terms of counts per minute per g of fresh weight):

no additives: 650,000;

8-hydroxyquinoline: 67,000;

a,a'-bipyridyl: 538,000;

NADH: 4,660,000;

NADPH: 4,110,000.

Information on benzene remaining unchanged is not reported. The results of the transformations by chloroplasts and enzyme preparations contain numbers for total radioactivity in terms of counts per minute per g of fresh weight with the factor 0.001. This would be lower by six orders of magnitude compared with test results obtained from whole leaves. So this prefix is presumed to be an error and changed to the factor 1,000.

**Source:**

German Rapporteur

**Test condition:**

1. Spinach plants were grown under sterile conditions in a nutrient solution. The benzene concentration in the air was 0.2 mmol/l (= 15.6 mg/l or 15.6 g/m<sup>3</sup>), the exposure time was 72 h at 22-26°C.

2. Light intensity was 10,000 erg/s/cm<sup>2</sup> = 1 mW/cm<sup>2</sup>, the phosphate buffer was 0.05 M at pH 6.5. Controls with

inactivated chloroplasts were run.  
3. The phosphate buffer was 0.1 M at pH 6.8.

**Test substance:** Radiolabeled benzene (3 g; 225.7 MBq/g) was diluted with unlabeled benzene to 30 g. After purification, the specific activity of labeled benzene was 1.76 MBq/mmol (= 22.5 MBq/g).

**Reliability:** (2) valid with restrictions

**Flag:** Risk Assessment

18-MAR-2004 (1177)

**Type:** plant

**Method:** Test system and exposure regime  
Cucumber and blackberry plants were maintained in a controlled environment (20 degrees C, 16 hr daylength etc) and exposed to either control air (passed through a charcoal filter to remove benzene) or ca. 1 mg/m<sup>3</sup> benzene for up to 3 months. Pot grown, outdoor dwarf apple trees were exposed on two 7 day occasions to filtered air or 1 or 9 mg/m<sup>3</sup> benzene within a controlled environment cabinet.

**Remark:** **Sampling and analysis**  
Three replicate samples of leaf disc, meristem or fruit tissue were taken from individual plants and placed inside a crimp-top headspace sampler. Samples were disintegrated mechanically and analysed by headspace GC/MS. Background (control) benzene concentrations were subtracted from all vegetation samples (mean = 1 ng/g tissue). Samples of chamber air were adsorbed onto ORBO-32 charcoaltubes, desorbed using dichloromethane and analysed by GC.

**Statistics**  
Applied, but methods not described.

Although under the controlled conditions of the study the concentration of benzene in plant tissues was found to generally increase following exposure to exogenous (air) sources, the authors conclude that ingestion of contaminated vegetation is not of significance unless high ambient levels are maintained for a long duration.

**Result:** (Results are presented graphically. Values presented below were obtained by interpolation.)

Blackberry leaf showed significant benzene accumulation (up to 1000 ng/g), whereas the level in fruit remained relatively stable (approx 10 ng/g). (Note: there was a single, unexplained peak value in fruit 55 days into the exposure period.)

There was little evidence of benzene uptake by cucumber leaves (barely detectable), whereas variable amounts were found in fruit (approx 2-25 ng/g) and meristem (up to approx 5 ng/g). Apple tree leaves showed variable uptake, with barely detectable amounts after the first exposure and approx 40 ng/g in old leaves, and approx 10 ng/g in new leaves, following the second (higher) exposure. The concentration in apple flowers after the first exposure was approx. 10 ng/g, while fruit contained approx. 35 ng/g after the second exposure.

**Reliability:** (2) valid with restrictions  
07-APR-2004 (225)

#### **4.9 Additional Remarks**

**Memo:** Physiological responses of a clam *Gafrarium divaricatum* (Gmelin) to xylene, benzene and gear oil-WSF.

**Remark:** The intertidal clams, *Gafrarium divaricatum* were exposed to sublethal concentrations of xylene, benzene and water soluble fractions of gear oil up to one month. Alterations in oxygen consumption and filtration rate of the *G. divaricatum* were noted after 96 h and one month exposure period. Pollutant stress was found to suppress the oxygen consumption and filtration rate of the clams when exposed for a long period.

Additional Comments: Benzene was evaluated at concentrations of 4.35 and 8.7 mg/L in this study. Oxygen consumption rates were found to be 0.455 and 0.509 ml O<sub>2</sub>/h/gm body weight in the clams, respectively, at the low and high benzene exposure levels, at 4 days, and were found to be 0.332 and 0.280 ml O<sub>2</sub>/h/gm body weight, respectively, at 30 days. Oxygen consumption rates in the control clams were 0.375 and 0.387 ml O<sub>2</sub>/h/gm body weight at 4 and 30 days, respectively. Elevation in filtration rates in the clams was more pronounced during the first 3 hrs at day 4, for both exposure concentrations of benzene, than during the same period at day 30, when, little if any effect in filtration rate was observed.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur

**Reliability:** (2) valid with restrictions  
29-JUL-2002 (1118)

**Memo:** Effect on the sexual reproduction of the marine red macroalga *Champia parvula*.

**Remark:** In a test developed in part by the EPA, short-term effects of benzene on sexual reproduction of the marine red macroalga *Champia parvula* was investigated. Unialgal stock cultures of both male and female gametophytes were grown for 3 weeks in sterile, filtered seawater with nutrients added. Thereafter, branches of female plants and one branch of a male plant producing spermatia were treated with benzene for 2 days (400 ml test solution in 500 ml screw-capped Erlenmeyer flasks, 22-24 degree C, salinity 7-7.5 g/l, 16:8 h light/dark cycle), followed by a 5-7 day recovery period for the females. Subsequently, the number of cystokarps per female were counted. Concentrations of benzene showing the following effects are nominal concentrations, no analytical monitoring to determine actual concentrations was performed. No sexual reproduction was observed at a benzene concentration of 73.6 mg/l. The EC50 value, i.e. the

**Source:** concentration decreasing the no. of cystokarps to 50% of control, was reported to be lower than 34.3 mg/l.  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Reliability:** (2) valid with restrictions (1131)  
18-MAR-2004

### **5.0 Toxicokinetics, Metabolism and Distribution**

**In Vitro/in vivo:** In vitro  
**Type:** Metabolism  
**Species:** mouse

**Method:** CYP2E1 knockout mice (129/Sv-CYP2E1<sup>tm1Gonz</sup>) and wild-type controls (12953/SrImJ) were used (no further details available).

Microsomal fractions were prepared from lung and liver tissue (pooled from 3 animals) using standard methods (differential centrifugation).

The incubation mixture contained microsomal protein (0.6 - 2.0 mg/ml), NADH, NADPH and <sup>14</sup>C-benzene (24 uM), and the experiments were conducted at 37 degrees C at pH 7.4. Diethyldithiocarbamic acid (300 uM: inhibits CYP2E1) and 5-phenyl-1-pentyne (5 uM: inhibits CYP2F2) were used as inhibitors. Metabolites were quantified using HPLC with UV detection.

**Result:** Students t test was used to assess statistical significance, after log transformation of the data.  
Metabolism by hepatic microsomal fractions  
Benzene metabolism in hepatic microsomes from CYP2E1 knockout mice was significantly less than in wildtype controls (96% decrease in production of total hydroxylated metabolites).

In wildtype mice, diethyldithiocarbamate (DDTC: a CYP2E1 inhibitor) decreased production of total hydroxylated metabolites by 80%, and 5-phenyl-1-pentyne (5PIP: a CYP2F2) by 27%. In knockout mice, DDTC decreased production of total hydroxylated metabolites by 70% while 5PIP gave only a minor (non-significant) decrease in metabolism (37% reduction).

Metabolism by pulmonary microsomal fractions  
Benzene metabolism in pulmonary microsomes from CYP2E1 knockout mice was less than in wildtype controls (45% decrease in production of total hydroxylated metabolites), but this difference was not as pronounced as that seen when liver fractions were compared.

Benzene metabolism in controls was inhibited to a similar degree by DDTC and 5PIP (approx. 70% reduction). DDTC decreased production of total hydroxylated metabolites by 58%, and 5PIP by 72%, in the pulmonary microsomal fraction from CYP2E1 knock-out mice.

**Source:** A.K. Mallett Surrey

**Conclusion:** The results confirmed that while CYP2E1 is the most important isozyme involved in benzene metabolism in the liver, CYP2F2 and CYP2E1 both an important role in the lung.

**In Vitro/in vivo:** In vivo

**Method:** Subjects  
6 adults (5 male, 1 female, age 29-48 yr) and 2 children (1 male, 1 female, ages 10 yr and 5 yr) were recruited. Exclusion criteria included chronic illness, use of medication and smoking.

Study design  
Adult subjects were studied over a 48 or 72 hr period, and children over 24 or 48 hr. Specific foods thought to have a high sorbic acid content (soft drink, baked goods, processed cheese) were ingested at lunchtime. Food consumed at other times was selected to contain a minimum of sorbic acid. A questionnaire was used to estimate exposure to other sources of sorbic acid and benzene.

Inhalation exposure to benzene was measured in 5 of the adults over 24 hr using a modification of NIOSH method 1500 (charcoal adsorption). Exposure in the other 3 subjects was by 24 hr passive vapor sampling. All samples were desorbed using acetone/carbon disulphide and quantified by GC analysis.

Urine was collected during the study period and stored frozen at -80 degrees until analysis for trans,trans-muconic acid (MA; HPLC-UV assay) and creatinine (colorimetric kit). The detection limit for MA was 16 ng/ml.

Statistics  
Data are tabulated or presented graphically, with no statistical analysis.

**Result:** MA levels in urine were increased from 27 - 50 ng/mg creatinine to 140 - 1752 ng/mg creatinine after ingestion of the soft drink. Levels remained elevated for approx. 4 - 10 hr.

The baseline level prior to eating two cakes was 19 - 91 ng/mg creatinine, increasing to 563 - 1514 ng/mg creatinine. Levels remained elevated for approx. 7 - 11 hr.

In a separate experiment, one adult ingested the soft drink and consumed the baked goods in the same meal, with a MA peak of 3131 ng/mg creatinine. Ingestion of a processed cheese sandwich resulted in a MA peak of 602 ng/mg creatinine.

There was no interference between MA and sorbic acid under the chromatographic conditions used.

Benzene exposures were found to be very low, and in a range <0.99 - 5.6 ppb.

**Source:** A.K. Mallett Surrey  
**Conclusion:** Sorbic acid, present in foods, is metabolized to

trans,trans-muconic acid (MA) and has the potential to interfere when MA is used as a biomarker for benzene exposure. The authors suggest sorbic acid ingestion may be responsible for the highly variable correlation between urinary MA and benzene exposure reported in the literature.

25-APR-2002

(1242)

**In Vitro/in vivo:** In vivo  
**Type:** Metabolism  
**Species:** rat

**GLP:** no data

**Method:** ANIMALS AND TREATMENTS  
Swiss-Webster mice (n=10) were given a single ip injection of 880 mg/kg bwt <sup>14</sup>C-labelled benzene (3.38 mCi/kg bwt) 2-days post-partum. Six dams continued to nurse their pups, while the other four were housed individually (with-out pups) in metabolism cages for urine collection over 24 hr. Pups from 4 four control litters (dams injected with vehicle only) were treated with benzene (as above) and returned to their mothers.

#### SAMPLE COLLECTION AND ANALYSIS

The animals were killed 5 hr or 24 hr following treatment with benzene and blood and liver sampled.

Urine was analysed immediately by HPLC (with or without treatment aryl suphatase/glucuronidase), the other samples were stored frozen (-80 degrees C) prior to analysis.

DNA was isolated from whole blood and liver and the amount of benzene-derived radioactivity determined by liquid scintillation counting.

#### STATISTICAL ANALYSIS

None specified.

**Result:** METABOLITE PROFILE IN LACTATING DAMS  
Five main metabolite fractions were isolated in urine from lactating dams 5hr after treatment with benzene:

A: 67%; uncharacterised\*

B: 5%; unknown

C: 9%; muconic acid

D: 12%; phenyl glucuronide

E: 7%; phenol

(\* there was insufficient material for full chemical characterisation, however the authors considered this probably contained hydroquinone, trihydroxybenzene and their glucuronides/sulphates + phenyl sulphates. Enzymic hydrolysis indicated the presence of 78% conjugates.)

#### METABOLITE PROFILE IN NURSING PUPS FROM DAMS TREATED WITH <sup>14</sup>C-BENZENE

The profile in pups suckling from mothers treated 24 hr previously with <sup>14</sup>C-benzene was qualitatively similar to

that of the mothers:

A: 73%  
B: 11%  
C: 3%  
D: 5%  
E: 8%

Metabolite B appeared to be higher in the pups, while C and D appeared higher in the dams. Enzymic hydrolysis of fraction A indicated that 48% of the radioactivity was present in conjugated form.

#### METABOLITE PROFILE IN SUCKLINGS TREATED DIRECTLY WITH <sup>14</sup>C-BENZENE

The urinary metabolite profile in pups 5hr after direct treatment with <sup>14</sup>C-benzene was qualitatively similar to that of treated dams, however a sixth metabolite (F) was also present:

A: 72%  
B: 1%  
C: <1%  
D: 7%  
E: 11%  
F: 8%

The lower levels of metabolites A, D and E in urine possibly reflected the earlier collection time of the sample. Metabolite F did not co-chromatograph with any authentic benzene metabolite. Enzymic hydrolysis of fraction A indicated the presence of 21% conjugates.

#### COVALENT BINDING OF <sup>14</sup>C-BENZENE TO DNA IN WHOLE BLOOD AND LIVER

Graphical data indicate that binding in whole blood was approx. 1.5-fold greater than in liver of pups treated directly with <sup>14</sup>C-benzene, while blood levels in directly treated pups were approx. 28-fold higher than liver levels. Blood levels in the treated dams were 1.9-fold higher than blood levels.

**Source:** A.K. Mallett Surrey

**Conclusion:** Bioactivation of benzene in the neonatal mouse after ip injection of high doses of benzene (880 mg/kg bwt) is comparable to that seen in the mother after similar treatment. Urinary metabolites profiles for sucklings exposed to benzene via lactation are also qualitatively similar.

04-MAR-2003

(555)

**In Vitro/in vivo:** In vitro  
**Type:** Absorption

**Method:** ANIMALS AND TREATMENTS  
Adult, male non-Swiss albino mice (n=3; 20-25 g), SD rats (n=2; 150-174 g) and NZ rabbits (n=6; 2-3 kg) were anaesthetised, and the lung perfused and removed following published methods (rabbit: Trela et al. (1988) Toxicol Appl

Pharmacol 96, 442-450; rats/mice: Skelly and Shertzer (1983) Exp Lung Res 5, 259-268). After removal, perfusion (via the pulmonary artery) was maintained using modified Krebbs-Henseleit buffer, the lung placed in an artificial thorax, and humidified compressed "breathing" air supplied via a tracheal cannula.

#### EXPOSURE VIA THE PULMONARY VASCULATURE

After the lungs were cleared of blood, <sup>14</sup>C-benzene was added to the perfusate, which was collected from the bottom of the artificial thorax via a sampling reservoir and recirculated. Samples were taken for analysis (HPLC) after perfusion for up to 30 minutes.

#### EXPOSURE TO BENZENE VAPOR VIA TRACHEA

Breathing air containing <sup>14</sup>C-benzene vapor was introduced into the tracheal cannula. Perfusate was collected (but not recirculated), and analysed (HPLC) for benzene and its metabolites. (Studies performed in mouse lung only)

#### STATISTICAL METHODS

No statistical methods were described or applied.

#### Result:

##### PERFUSED RABBIT LUNG

Benzene (initial concentration 50 uM) disappeared rapidly from perfusate recirculated via the pulmonary artery (decreased 50% in approx. 5 min), and a semi-log plot showed that this was a first order process. The amount of phenol present in the perfusate increased rapidly over 9 min, and remained relatively constant thereafter. Phenyl sulphate was also present, but the HPLC method used could not separate this from a contaminant peak present in the benzene.

##### PERFUSED RAT LUNG

Benzene (205 uM) rapidly disappeared from the arterial perfusate (decreased by approx. 50% in 7 min), again via an apparently first order process. Phenol was formed quickly during the first 5 min of perfusion, but the rate slowed thereafter. No other metabolites were formed (below limit of detection).

##### PERFUSED MOUSE LUNG

Three substrate concentrations (55, 120 and 200 uM benzene) were used in these arterial perfusion studies. Disappearance of benzene from the recirculating perfusate was rapid at all 3 concentrations (decreased by 50% in approx. 3-5 minutes). Removal was first order over the first 10 minutes of perfusion (based upon semi-log plots), but deviated from first order thereafter. Phenol was the main metabolite formed: this was produced in similar amounts at the two lower concentrations (no dose relationship), while larger amounts (approx. 4-5 fold more) were present when 200 uM benzene was used. Phenyl sulphate was also present, but could not be quantified accurately (see above).

In experiments where benzene vapor was present in the air, its concentration in the non-recirculating perfusate

increased in parallel with the concentration in the air. During the final 10 min of the study, the concentration of benzene in air was approx. 175 ppm, while the concentration in the perfusate was approx. 1.5-2.1 uM. Phenol was also present in the perfusate, demonstrating pulmonary metabolism of benzene. The rate of formation increased gradually over the first 10 min, then remained relatively constant (8-14 pmol/min) for the remainder of the study.

**Source:** A.K. Mallett Surrey

**Conclusion:** Results from isolated perfused lung experiments demonstrate that the lung can metabolise benzene in a substrate concentration-dependent manner. Metabolism occurred following exposure both via the vasculature and via air.

04-MAR-2003

(907)

**In Vitro/in vivo:** In vivo  
**Type:** Toxicokinetics  
**Species:** mouse

**Method:** ANIMALS AND TREATMENT  
Tg.AC or FVB (parental strain) mice (age and sex not specified) were given a single dose of 220 mg/kg bwt of <sup>14</sup>C-benzene (17.73 mCi/mmol) via oral gavage or intradermal injection. Propylene glycol was used as vehicle. Blood, liver, spleen, lung, kidney, stomach, large- and small intestine, epididymal fat pads, femur and the skin around the dosing site were sampled 10, 30, 60, 90, 120 or 240 min post-dosing (under anaesthesia, method not stated).

ANALYSIS AND CALCULATION OF KINETIC CONSTANTS  
Organs and femoral bone marrow were homogenised in 50% methanol (with ascorbic acid and zinc sulphate) prior to removal of protein (centrifugation) and analysis by HPLC. The area under the curve (AUC) was calculated for benzene and each of its metabolites in each organ, and elimination (Kel) and absorption (Ka) rate constants calculated.

STATISTICAL METHODS  
No statistical analysis was applied.

**Remark:** The authors note that French et al (Carcinogenesis, in press, 2000) reported the occurrence of granulocytic leukaemia in Tg.AC mice following dermal application of benzene for 26 wk. No increase was observed following oral treatment, nor were FVB mice susceptible after treatment via either route. They suggest that strain-specific differences in pharmacokinetics are responsible for this difference.

**Result:** In addition to unmetabolised benzene, phenol, phenol sulphate, phenol glucuronide, hydroquinone sulphate (HQS), hydroquinone glucuronide (HQG), muconic acid (MA) and phenylmercapturic acid were detected in most organs, regardless of the strain or route of exposure.

Absorption constants (Ka values) were lower following intradermal injection than after oral administration for the

majority of tissues, however there were no major differences between the strains. Little difference was noted in Kel for each of the different groups.

Comparison of AUC values for HQS and HQG in the various tissues showed that values were higher after intradermal administration than after oral treatment. The AUC for MA, in contract, was higher in the tissues after oral treatment.

**Source:** A.K. Mallett Surrey

**Conclusion:** Strain-specific differences in benzene pharmacokinetics may influence the carcinogenic (leukaemogenic) activity of benzene in Tg.AC and FVB mice.

04-MAR-2003

(519)

**In Vitro/in vivo:** In vitro  
**Type:** Absorption

**Method:** Samples of human skin (anterior thigh) were obtained at autopsy from a single donor. Stratum corneum was separated from full thickness skin and dried over silica gel. Viable epidermis and dermis were separated and, if not used immediately, wrapped in aluminium foil (to preserve hydration) and stored refrigerated.

Tissue was weighed and placed into a 2 ml screw capped vial (Teflon-lined cap) containing <sup>14</sup>C-benzene (0.045 uCi) and a known volume of water, blood substitute (MEM Eagle's medium with Earle's BSS and 4% BSA) or benzene. The mixture was shaken briefly and left overnight in a refrigerator to equilibrate. Liquid and tissue phases were separated, the tissue digested, and radioactivity in both phases counted.

**Result:** The following partition coefficients were obtained:

Blood substitute: viable epidermis 2.4 (n=5)  
Blood substitute: dermis 11.2 (n=4)  
Benzene: stratum corneum 4.2 (n=5)  
Whole skin: blood substitute 2.2 (n=1)  
Benzene: water 109, 126 (n=2)  
Benzene: blood substitute 55, 59 (n=2)

**Source:** A.K. Mallett Surrey

19-MAR-2004

(1037)

**In Vitro/in vivo:** In vivo  
**Type:** Toxicokinetics  
**Species:** mouse

**Method:** ANIMALS AND TREATMENTS  
In the dose-response portion of this study, male B6C3F1 mice (30 g) were given doses of <sup>14</sup>C benzene in the range 5 ng/kg bwt to 500 mg/kg bwt (6 dose levels). The amount of radioactivity was adjusted with unlabelled benzene such that

all animals received 0.1 uCi, administered in 200 ul corn oil. Animals were killed (carbon oxide) 0-48 hr later.

In toxicokinetic investigations, mice received 50 ug <sup>14</sup>C benzene (as above).

#### SAMPLE COLLECTION AND HPLC ANALYSIS

Blood was collected (cardiac puncture) into heparinised tubes, centrifuged and plasma snap-frozen (liquid nitrogen) and stored at -35 degrees C. Liver was sampled and immediately placed on dry ice and stored frozen. Bone marrow was collected from femur and humerus, snap frozen and stored. Metabolites were separated using HPLC with UV detection (Sabourine et al. (1988) Toxicol Appl Pharmacol 94, 128). Results were normalised to recoveries of internal standard and expressed as fmol/ml HPLC effluent.

Urine was collected (method not specified) for 24 hr post-exposure and stored in the dark in presence of ascorbic acid (antioxidant) until analysis. Metabolites were separated by HPLC (Sabourine et al. (1988) Anal Biochem, 170, 316) with UV detection.

#### ISOLATION OF DNA AND PROTEIN FROM TISSUE SAMPLES

DNA was isolated from liver or bone marrow after solubilisation and RNase treatment followed precipitation with isopropyl alcohol. This procedure gave approx. 100 ug DNA per 100 mg of starting tissue. Protein was precipitated with perchloric acid after solubilisation, and protein content determined using the Bradford microassay.

#### ACMS ANALYSIS

The radiocarbon content of samples, DNA extracts, protein extracts and metabolite fractions was determined using accelerator mass spectrometry (AMS) after converting dried samples to graphite and subsequent analysis using published procedures (Turtletaub et al. (1993) Postlabeling Methods for Detection of DNA Adducts, IARC, Lyon, pp 293-300).

Comment: AMS measures the ratio <sup>14</sup>C relative to <sup>13</sup>C, which was then normalised to <sup>14</sup>C/<sup>12</sup>C using a standard carbon source. The ratios were converted to mass of <sup>14</sup>C benzene based upon the specific activity of the benzene after correction for background.

#### STATISTICAL METHODS

Dose-response data were log-transformed to stabilise variances across the several orders of magnitude covered by the data. Z tests were used to examine for differences between AUCs for adduct formation.

#### Result:

##### DOSE-DEPENDENT METABOLISM

HPLC-accelerator mass spectrometry demonstrated the dose-dependent presence of 3 major radiocarbon peaks (phenyl sulphate, muconic acid and an uncharacterised peak) and 2 minor metabolites (phenyl glucuronide, hydroquinone sulphate) in urine from mice given 50 ng/kg to 500 mg/kg benzene by single i.p. injection. (Free catechol and free

hydroquinone were also present, but not dose-dependent.) Although dose-dependent, amounts of the 3 major metabolites formed at lower exposures (5-500 ng/kg bwt) was proportionately less than at higher doses. The relative amount of each metabolite remained approx. constant over the treatment range and followed the order:

unidentified > phenyl sulphate > muconic acid > phenyl glucuronide > hydroquinone sulphate.

Comment: The unknown peak present in urine in these low-dose studies has not been reported by other investigators using higher benzene treatment; attempts to establish its identity were unsuccessful.

In liver, muconic acid, catechol and hydroquinone were formed in a dose-dependent manner (phenol present but not dose-dependent). In plasma and bone marrow, none of the metabolites were found to be dose-dependent.

#### TOXICOKINETICS OF LOW-DOSE BENZENE METABOLISM

AUCs showed that hydroquinone (149 fmol) and muconic acid (56 fmol) were the major metabolites present in mouse liver over 48 hr following administration of 5 ug benzene /kg bwt (i.p.), with comparatively minor amounts of catechol (2 fmol) and phenol (3 fmol).

A similar metabolite profile was present in bone marrow, however AUCs were much lower and muconic acid predominated (6, 16, 1 and 3 fmol for hydroquinone, muconic acid, catechol and phenol, respectively).

Minor amounts of metabolites were present in plasma, with marginally more hydroquinone present than for the other 3 metabolites (1.8, 0.6, 0.3, 0.3; order as previous).

Comment: The AUC data were highly variable; the authors suggest biological variation and sampling artefacts may have been responsible (- AMS methodology considered highly reliable, low inter-sample variation).

#### RELATIONSHIP BETWEEN DOSE AND ADDUCT FORMATION IN MICE

Graphical results of log-transformed data show that protein and DNA adduct levels in liver and bone marrow at 1 hr and 12 hr increased in a dose dependent manner following administration of benzene at doses of 5 ng/kg to 500 mg/kg (i.p.).

Levels of both adducts in liver (but not bone marrow) appeared to plateau at doses >50 mg/kg bwt and tended to be higher 1 hr post-dosing.

In bone marrow, adduct levels were equivalent at both time points for protein and higher at 12 hr post-dosing for DNA.

Comment: In very general terms, low doses of benzene (ng-ug range) appeared to yield  $10^1$  to  $10^2$  pg bound benzene

equivalents per g tissue in DNA from liver and bone marrow, respectively; DNA from mice given high doses (500 mg/kg bwt) gave around  $10^6$  to  $10^7$  pg equivalents per g liver or bone marrow.

**Source:** A.K. Mallett Surrey

**Conclusion:** These results demonstrate a dose-dependent increase in presence of benzene metabolites in urine following i.p. administration to mice over the range 5 ng/kg bwt to 500 mg/kg bwt. The dose-response curve at lower doses (5-500 ng/kg bwt) was flatter than at higher doses but still demonstrated detectable metabolic conversion. DNA and protein adducts were also detected in liver and bone marrow over this same range of treatments and increased in a dose-dependent manner.

08-APR-2004

(1171)

## 5.1 Acute Toxicity

### 5.1.1 Acute Oral Toxicity

**Type:** LD50  
**Species:** rat  
**No. of Animals:** 10  
**Doses:** 88, 810, 1870 mg/kg bw  
**Value:** = 810 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** At high doses of 1870 mg/kg bw, animals developed tremors and convulsions and many died within 20 min. At 88 mg/kg bw, slight CNS depression occurred but no deaths. Guidelines followed not specified; groups of ten non-fasted or 24-hr fasted animals.

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Test substance:** Test substance was laboratory reagent grade.

**Flag:** Risk Assessment

07-JUL-2005

(243)

**Type:** LD50  
**Species:** rat  
**Value:** = 10000 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of five non-fasted males; stomach tube; observed for 14 days. Doses not dated.  
The LD50 value was reported as 10,020 mg/kg bw (range 7,380-14,855 mg/kg bw).

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1062)

**Type:** LD50  
**Species:** rat  
**Doses:** 2000, 2990, 4470, 6690, 10000 mg/kg bw (10 rats/group) 3000, 4250, 6000, 8460, 11920 mg/kg bw (20 rats/group)  
**Value:** = 5960 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Guidelines followed not specified; groups of ten or 20 animals; stomach tube; observed for 7 days.  
The LD50 value was reported as 5960 mg/kg bw (range 5080-7000 mg/kg bw). Hind-limb paralysis, petechial bleeding of the urinary tract, eyes and nose, mild acute gastritis and stripping of the epithelial lining of the glandular portion of the stomach were seen.

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1261)

**Type:** LD50  
**Species:** rat  
**Value:** = 3400 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of six to twelve rats of both sexes/dose of newborn (24-48-hr-old) and immature (14-day-old) rats and groups of six males of young adult and older adult rats; stomach tube; undiluted; observed for 7 days.

No testdoses reported.

The LD50 values for the 14-day-old, young adult and older adult groups were 1758-5010, 2549-4219 and 3516-6856 mg/kg bw respectively. Oral LD50 values were calculated as 3400 mg/kg for 14-day old rats, 3800 mg/kg for young adult rats, and 5600 mg/kg for older rats. A rough approximation of the acute LD50 in the newborn rats found volumes of about 1 ml/kg bw [about 880 mg/kg bw] to be fatal.

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Test substance:** Test substance was analytical grade laboratory reagent meeting ACS specifications.

**Flag:** Risk Assessment

07-JUL-2005

(620)

**Type:** LD50  
**Species:** rat  
**Value:** ca. 5600 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Guidelines followed not specified; 25 male animals; stomach tube; benzene given either undiluted or as an olive-oil or corn-oil solution emulsified with a 5-10% aqueous solution of gum arabic; observed for 14 days. No data on doses or animals/dose, no data on clinical signs, and no more data on necropsy are documented.

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Test substance:** 99.98% pure.

**Flag:** Risk Assessment

07-JUL-2005

(1264)

**Type:** LD50  
**Species:** rat  
**Value:** = 930 mg/kg bw

**Method:** other: see reference  
**Year:** 1965  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (1152)

**Type:** LD50  
**Species:** rat  
**Value:** = 810 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** method: groups of ten fasted male Sprague-Dawley rats;  
geometrically graded doses.  
Reliability: 3 (not valid)  
Documentation insufficient for assessment  
one sex; no pathology; no data about number of doses, post  
exposure observation period, mortality rate, time to death  
after dosing  
results: 95% confidence limits 690-950; at high doses of  
1870 mg/kg bw, animals developed tremors and convulsions and  
many died within 20 min.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Test substance was laboratory reagent grade.  
06-JAN-1997 (244)

**Type:** LD50  
**Species:** rat  
**Value:** = 3340 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** methods: 6 male young adult Sprague-Dawley rats per group; additional experiments with a) 6 male older adult rats/group, b) 6-12 male and female newborn rats (24-48-h-old) per group and c) 6-12 14-days-old rats of both sexes per group; stomach tube; undiluted; observed for 7 days.

Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions one sex; short observation period; no data about clinical examination, mortality rate, time of death after dosing and pathology  
results: 95% confidence limits 2550-4220 mg/kg; LD50 values in a) 4920 (3520-6860) mg/kg and in b) 2990 (1760-5010) mg/kg; c) rough approximation of the acute LD50 in the newborn rats found volumes of about 1 ml/kg bw [about 880 mg/kg bw] to be fatal.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Test substance was analytical grade laboratory reagent meeting ACS specifications.

06-JAN-1997

(620)

**Type:** LD50  
**Species:** rat  
**Value:** ca. 5600 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** methods: guidelines followed not specified; 25 male animals; stomach tube; benzene given either undiluted or as an olive-oil or corn-oil solution emulsified with a 5-10% aqueous solution of gum arabic; observed for 14 days.

Reliability: 3 (not valid)  
Documentation insufficient for assessment  
no data about number of rats per group, clinical and pathological examination, assesement of toxicity in the other sex, doses

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** 99.98% pure.

06-JAN-1997

(1264)

**Type:** LD50  
**Species:** rat  
**Value:** = 5960 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Guidelines followed not specified; groups of 10 (pilot study) or 20 Spreague-Dawley Cobb rats; 5 doses tested; stomach tube; observed for 7 days.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions no data about the test substance or time of death after dosing; short observation period after dosing; one sex  
The LD50 95% confidence limits were reported as 5080-7000 mg/kg bw. Hind-limb paralysis, petechial bleeding of the urinary tract, eyes and nose, mild acute gastritis and stripping of the epithelial lining of the glandular portion of the stomach were seen.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1261)

**Type:** LD50  
**Species:** rat  
**Value:** = 10020 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** 95% confidence limits 7,380-14,855 mg/kg.  
Groups of five non-fasted male Carworth-Wistar rats; stomach tube; observed for 14 days;  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions one sex; ; no data about clinical examination, time of death after dosing, mortality rate and pathology;

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1062)

**Type:** LD50  
**Species:** mouse  
**Value:** 8438 mg/kg bw

**Method:** other  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Reliability: 2 (eingeschaenkt gueltig)  
Rahmendaten liegen vor  
Zur Bestimmung der akuten Toxizitaet wurden nur weibl. Tiere verwendet.

**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (78)

**Type:** LD50  
**Species:** mouse  
**Value:** 8438 mg/kg bw  
  
**Method:** other  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4  
  
**Remark:** Zur Bestimmung der akuten Toxizitaet wurden nur weibl. Tiere verwendet.  
**Source:** BASF AG Ludwigshafen  
**Reliability:** (2) valid with restrictions  
 Rahmendaten liegen vor  
 08-NOV-1995 (78)

**Type:** LDLo  
**Species:** human  
**Value:** = 50 mg/kg bw  
  
**Method:** other: see reference  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data  
  
**Source:** REPSOL PETROLEO, S.A. MADRID  
 13-DEC-1996 (399)

### 5.1.2 Acute Inhalation Toxicity

**Type:** LC50  
**Species:** rat  
**Exposure time:** 4 hour(s)  
**Value:** = 13700 ppm  
  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS  
  
**Remark:** Method approximates to OECD Test Guide-line, 403 but females only were tested. Groups of ten female animals; observed for 2 wk; animals dying during exposure and those killed at end of study subjected to necropsy. Number of doses and dose concentrations were not given.  
 The LC50 value was reported as 13,700 ppm (converts to 44.7 mg/l) with a range of 13,050-14,380 ppm (converts to 42.5-46.9 mg/l). Death appeared to be caused by a depression of the CNS. These animals had increased lung and liver weights, lung and liver congestion and an increased number of vacuolated hepatocytes in the liver.  
**Source:** BP Chemicals Ltd LONDON  
 German rapporteur  
**Test substance:** Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% was tested.  
**Flag:** Risk Assessment  
 07-JUL-2005 (310)

**Type:** other  
**Species:** rat  
**Sex:** male  
**No. of Animals:** 6  
**Exposure time:** 4 hour(s)  
**Value:** < 16000 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Four of the six males died following exposure to 16000 ppm (52.2 mg/l). Five minutes were stated as the maximum time of concentrated vapour inhalation without deaths. No more data were given.

Groups of six males; observed for 14 days.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1062)

**Type:** LC50  
**Species:** mouse  
**No. of Animals:** 182  
**Doses:** 4980, 7490, 8330, 9280, 10200, 10450, 10950, 11540, 12430, 14600 ppm (15.9, 23.9, 26.6, 29.6, 32.2, 33.3, 34.9, 36.8, 39.6, 46.5 mg/l)  
**Exposure time:** 7 hour(s)  
**Value:** = 10400 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** other TS: purity 99.5%

**Remark:** method: male and female Swiss mice, concentration analysed by interferometric determination, 16-20 mice per group, 10 concentrations tested, post exposure observation period up to 4 weeks but deaths occurred mostly within 8 h after start of exposure.  
results: LC50 after 1 h post exposure observation period 10400 ppm, converted to 31.79 mg/l; 95% confidence limits 9180-10860ppm; symptoms: restlessness, tremor, muscular twitching, S-shaped tail, changes in respiration, incoordination, narcosis; histopathology: congestion of lungs and kidneys, nuclear fragments in spleen follicles.  
Reliability: 1

Comparable to guideline study  
LC50 not determined for each sex

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1102)

**Type:** LC50  
**Species:** rat  
**Value:** > 40000

**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of eight male animals; removed from chamber when convulsions or respiratory arrest seen and returned as soon as they recovered; necropsy performed on CNS after 24 hr. Three of the eight animals died following five 25-35 min exposures to 40,000 ppm (130.4 mg/l). Local irritation, depression of CNS and quivering or twitching were noted. Necropsy revealed no gross lesions other than those attributable to local irritation of the respiratory tract and no specific damage to the CNS.

**Source:** BP Chemicals Ltd LONDON

13-DEC-1996

(384)

**Type:** LC50  
**Species:** rat  
**Exposure time:** 7 hour(s)  
**Value:** = 10000 ppm

**Method:** other: see ref.

**Year:** 1960

**GLP:** no data

**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(1153)

**Type:** LC50  
**Species:** rat  
**Exposure time:** 4 hour(s)  
**Value:** = 13700 ppm

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Method approximates to OECD Test Guide-line, 403 but females only were tested. Groups of ten female animals; observed for 2 wk; animals dying during exposure and those killed at end of study subjected to necropsy. Chamber concentration monitored.

Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions one sex; no data about concentrations tested, mortality rate The LC50 value was reported as 13,700 ppm (converts to 44.7 mg/l) with 95% confidence limits of 13,050-14,380 ppm (converts to 42.5-46.9 mg/l). Death appeared to be caused by a depression of the CNS. These animals had increased lung and liver weights, lung and liver congestion and an increased number of vacuolated hepatocytes in the liver.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Reagent grade thiophene-free benzene containing no

06-JAN-1997 contaminants above a concentration of 0.05% was tested. (310)

**Type:** other  
**Species:** rat  
**Value:** > 40000 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of eight male animals; removed from chamber when convulsions or respiratory arrest seen and returned as soon as they recovered; necropsy performed on CNS after 24 hr. Reliability: 3 (not valid)  
Unsuitable test system  
no LC50 determination  
Three of the eight animals died following five 25-35 min exposures to 40,000 ppm (130.4 mg/l). Local irritation, depression of CNS and quivering or twitching were noted. Necropsy revealed no gross lesions other than those attributable to local irritation of the respiratory tract and no specific damage to the CNS.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (384)

**Type:** LCLo  
**Species:** human  
**Exposure time:** 5 minute(s)  
**Value:** = 2000 ppm  
**Method:** other: see ref.  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (399)

**Type:** other

**Remark:** Meerschweinchen, Kaninchen und Katzen wurden gegenueber Benzoldaempfen exponiert. (Die Dosierungen lagen zwischen 14 und 146 mg.) Die Vergiftungssymptome waren bei allen Spezies gleich. Die Benzoldaempfe fuehrten ohne nennenswertelokale Reizungen unter Kraempfen (Zuckungen) zu Narkose und bei laengerdauernder Exposition zu Atemlaehmung und Tod.  
Wurde waehrend der Narkose die Exposition abgesetzt erholtensich die Tiere rasch und vollstaendig.  
Reliability: 3 (ungueltig)  
entspricht nicht heutigen Kriterien

**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (80)

**Type:** other**Remark:** Meerschweinchen, Kaninchen und Katzen wurden gegenueber Benzoldaempfen exponiert. (Die Dosierungen lagen zwischen 14 und 146 mg.) Die Vergiftungssymptome waren bei allen Spezies gleich. Die Benzoldaempfe fuehrten ohne nennenswertelokale Reizungen unter Kraempfen (Zuckungen) zu Narkose und bei laengerdauernder Exposition zu Atemlaehmung und Tod.

Wurde waehrend der Narkose die Exposition abgesetzt erholtensich die Tiere rasch und vollstaendig.

**Source:** BASF AG Ludwigshafen**Reliability:** (3) invalid  
entspricht nicht heutigen Kriterien

08-NOV-1995

(80)

### 5.1.3 Acute Dermal Toxicity

**Type:** LD50  
**Species:** guinea pig  
**Value:** > 8260 mg/kg bw**Method:** other  
**GLP:** no data  
**Test substance:** other TS: laboratory reagent grade**Remark:** method: 4 male Hartley derived guinea pigs per group, 3 doses tested, benzene applied undiluted on a 1 inch square cellulose pad fixed to the clipped, intact or abraded abdominal or dorsal skin.  
No test doses reported.  
results: no differences between abraded and intact skin.  
Reliability: 3 (not valid)  
Documentation insufficient for assessment  
one sex, exposure and observation period not specified, 4 instead of 5 animals/dose, no data about mortality (time to death after dosing), no clinical examination and no pathology reported**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur**Flag:** Risk Assessment

07-JUL-2005

(970)

**Type:** LD50  
**Species:** rabbit  
**Value:** > 8260 mg/kg bw**Method:** other: Regulations 21 CFR 191.10  
**GLP:** no data  
**Test substance:** other TS: laboratory reagent grade**Remark:** method: 4 albino rabbits of both sexes per group; at least 3 doses tested; abraded skin.  
No test doses reported.  
Reliability: 2 (valid with restriction)

Test procedure in accordance with national standard methods with acceptable restrictions  
4 animals per group; used guidelines not available, but LD50 value >2000 mg/kg which is recommended in the limit test OECD 402

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(970)

**Type:** LD50

**Species:** rabbit

**Value:** > 9400 mg/kg bw

**Method:** other: see ref.

**Year:** 1965

**GLP:** no data

**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(1151)

**Remark:** No relevant data identified from the literature searched.

**Source:** BP Chemicals Ltd LONDON

13-DEC-1996

#### **5.1.4 Acute Toxicity, other Routes**

**Type:** LD50

**Species:** rat

**Route of admin.:** i.p.

**Value:** 2450 - 3530 mg/kg bw

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Groups of eight female rats; 50-50 (v/v) solution in mineral oil; necropsy on all animals dying during study and on all survivors killed after 14 days.

The LD50 value was reported as 2940 mg/kg bw (range 2450-3530 mg/kg bw). Animals died from CNS depression. No significant histologic damage observed in the lungs of any animal.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% was tested.

13-DEC-1996

(310)

**Type:** LD50  
**Species:** rat  
**Route of admin.:** i.p.  
**Value:** = 2890 other: ug/Kg

**Method:** other: see ref.  
**Year:** 1977  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (106)

**Type:** LD50  
**Species:** rat  
**Route of admin.:** i.p.  
**Value:** = 2940 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** 95% confidence limits 2450-3530 mg/kg bw. Animals died from CNS depression. No significant histologic damage observed in the lungs of any animal. Groups of eight female rats; 50-50 (v/v) solution in mineral oil; necropsy on all animals dying during study and on all survivors killed after 14 days.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% was tested.  
06-JAN-1997 (310)

**Type:** LD50  
**Species:** mouse  
**Route of admin.:** i.p.  
**Value:** 245 - 370 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** The LD50 value was reported as 300 mg/kg bw (range 245-370 mg/kg bw). No further details were available.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (625)

**Type:** LD50  
**Species:** mouse  
**Route of admin.:** i.p.  
**Value:** = 340 mg/kg bw

**Method:** other : no data  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (34)

**Type:** LD50  
**Species:** mouse  
**Route of admin.:** i.p.  
**Value:** = 298 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** 95% confidence limits 245-370 mg/kg; LD50 of benzene dissolved in DMSO 81 mg/kg; no further details were available.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (625)

**Type:** other  
**Species:** mouse  
**Route of admin.:** s.c.  
**Value:** 88 - 2200 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of males administered 88, 440 or 2200 mg/kg bw; 50% (v/v) benzene - olive oil solution; uptake of radioiron (<sup>59</sup>Fe), given as ferrous citrate, into the circulating erythrocytes followed. No effect on iron uptake after 72 hr was seen with the lowest dose level or with the mid- and high-doses when administered 1 and 12 hr before iron administration. When 440 or 2200 mg/kg bw were given 24 hr before the iron, the amount of iron incorporated into the circulating erythrocytes after 72 hr was dose-dependently reduced. When determined over a 24-hr period, iron incorporation was reduced dose-dependently when the mid- and high-benzene doses were given 24 and 48 hr before the iron dose but not at 1, 12 and 72 hr before, showing complete recovery of benzene's bone marrow suppression effect.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (676)

**Type:** other  
**Species:** mouse  
**Route of admin.:** s.c.  
**Value:** 88 - 2200 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of males administered 88, 440 or 2200 mg/kg bw; 50% (v/v) benzene - olive oil solution; uptake of radioiron (<sup>59</sup>Fe), given as ferrous citrate, into the circulating erythrocytes followed.  
No effect on iron uptake after 72 hr was seen with the lowest dose level or with the mid- and high-doses when administered 1 and 12 hr before iron administration. When 440 or 2200 mg/kg bw were given 24 hr before the iron, the amount of iron incorporated into the circulating erythrocytes after 72 hr was dose-dependently reduced. When determined over a 24-hr period, iron incorporation was reduced dose-dependently when the mid- and high-benzene doses were given 24 and 48 hr before the iron dose but not at 1, 12 and 72 hr before, showing complete recovery of benzene's bone marrow suppression effect.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(676)

## **5.2 Corrosiveness and Irritation**

### **5.2.1 Skin Irritation**

**Species:** rabbit  
**Exposure Time:** 4 hour(s)  
**Result:** irritating  
**EC classificat.:** irritating

**Method:** OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"  
**Year:** 1981  
**GLP:** no data  
**Test substance:** no data

**Remark:** method: 6 shaved New Zealand White rabbits, 4 h occlusive application under exposure chamber of 6 cm<sup>2</sup>; scoring criteria according to Draize, J.H. et al., JPET 82, 377-390 (1944); classification in compliance with 79/831/EEC, Annex VI, part IID.  
results: mean score of erythema 1, 24, 48, 72, 144 h after end of application 1.0, 2.0, 2.2, 2.4, 3.0, respectively; mean score of edema 2.2 1 h after end of application (other observation periods = 0)  
Reliability: 1 (valid)  
Guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment

07-JUL-2005

(577)

**Species:** rabbit  
**Concentration:** 100 %  
**Result:** irritating

**Method:** other  
**Year:** 1941  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Slight-moderate irritation (i.e. perceptible-definite erythema) and moderate necrosis (i.e. development of oedema and superficial necrosis resulting in a chapped appearance and exfoliation of large patches of skin). Blistering was seen, particularly when the material was confined. The method of Adams E.M. et al. Indust. Med. 2, 1, 1941 was followed. Neat material applied 10-20 times to the ear and abdomen (bandaged; type not specified) of an unspecified number of animals over a 2-4 wk period. Animals were observed daily and weighed weekly.

**Source:** BP Chemicals Ltd LONDON  
German rapporteur

**Test substance:** 99.98% pure.  
**Flag:** Risk Assessment

07-JUL-2005

(1264)

**Species:** rabbit  
**No. of Animals:** 5

**Method:** Primary skin irritation on rabbits is recorded in a 10-grade ordinal series and is based upon the severest reaction that develops on the clipped skin of each of five albino rabbits within 24 hours of the uncovered application of 0.01 ml of undiluted sample.

**Result:** Primary skin irritation graded 3 out of a scale of 10 was recorded as the severest reaction.  
No more data were given.

**Source:** German rapporteur  
**Flag:** Risk Assessment

07-JUL-2005

(1062)

**Species:** rabbit  
**Result:** slightly irritating  
**EC classificat.:** irritating

**Method:** other: see reference  
**Year:** 1962  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996

(25)

**Species:** rabbit  
**Result:** moderately irritating  
**EC classificat.:** irritating

**Method:** other: see ref.  
**Year:** 1986  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (908)

**Species:** rabbit  
**Result:** irritating

**Method:** other  
**Year:** 1941  
**GLP:** no data  
**Test substance:** other TS

**Remark:** methods: The method of Adams E.M. et al. Indust. Med. 2, 1, 1941 was followed. Neat material applied 10-20 times to the ear and abdomen (bandaged; type not specified) of an unspecified number of animals over a 2-4 wk period. Animals were observed daily and weighed weekly.  
Reliability: 3 (not valid)

Significant methodological deficiencies  
No data about number of animals tested, type of patch, dose level, observation period; single exposure (4 h) recommended  
results: slight-moderate irritation (i.e. perceptible-definite erythema) and moderate necrosis (i.e. development of oedema and superficial necrosis resulting in a chapped appearance and exfoliation of large patches of skin).  
Blistering was seen, particularly when the material was confined. No abnormal behavior or body weight loss.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** 99.98% pure.  
06-JAN-1997 (1264)

### **5.2.2 Eye Irritation**

**Species:** rabbit  
**Result:** moderately irritating

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Method approximates to OECD Test Guide-line, 405. Two drops of liquid [presumably neat and approximately 100 ml] placed onto right eyeball [exact area not specified] and visual observations of irritation and internal and external corneal injury [no further details available] were made 3 min, 1 hr and 1, 2 and 7 days after treatment.  
Moderate conjunctival irritation (i.e. inflammation and slight swelling of the eyelids) and very slight transient

corneal injury (i.e. questionable or just perceptible superficial necrosis in very small areas of the cornea) were recorded.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Test substance:** 99.98% pure.

**Flag:** Risk Assessment

07-JUL-2005

(1264)

**Species:** rabbit

**Method:** other

**Year:** 1946

**Method:** Applying a method developed by Carpenter and Smyth (Carpenter and Smyth, 1946), corneal necrosis graded 3 (out of a scale of 10) was reported for benzene. This test method uses 0.005 ml of the undiluted material which is applied to the center of the corneas of usually 5 rabbits while the lids are retracted. About 1 minute later, the lids are released. Eighteen to 24 hours later, the eye is examined and the injury scored according to a specific scoring system for corneal necrosis. The individual numerical scores of each eye treated with a chemical are added together and then divided by the number of eyes to obtain the score of the injury caused by the treatment. A score level of 5.0 is selected as representative of severe injury. This figure corresponds to necrosis, visible only after staining and covering about 3/4 of the surface of the cornea; or a more severe necrosis covering a smaller area. Guided by the result of the scoring after instillation of 0.005 ml of the substance and a table of standardised injury grades, additional applications are made (using higher volumes of the substance or using dilutions of the substance) until the chemical can be assigned to one of the recognised grades for corneal necrosis.

**Result:** In a large list of test results is stated for benzene (no data on purity), that a test according to this Carpenter and Smyth method revealed corneal necrosis graded 3. Injury grade 3 is defined as follows: 0.1 ml of the undiluted substances gives injury of up to 5.0 points or 0.5 ml gives injury over 5.0 points. No data on healing time are documented.

**Source:** German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1062)

**Species:** rabbit  
**Result:** highly irritating  
**EC classificat.:** risk of serious damage to eyes

**Method:** other: see ref.  
**Year:** 1986  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (25)

**Species:** rabbit  
**Result:** moderately irritating  
**EC classificat.:** irritating

**Method:** other: see ref.  
**Year:** 1956  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (24)

### 5.3 Sensitization

**Remark:** No relevant data identified from the literature searched.  
**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

**Remark:** No relevant data identified from the literature searched.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

#### **5.4 Repeated Dose Toxicity**

**Species:** mouse **Sex:** male  
**Strain:** other: DBA/2J  
**Route of administration:** inhalation  
**Exposure period:** 5 days  
**Frequency of treatment:** 6 h/d  
**Post exposure period:** 1 day and 5 days  
**Doses:** 10, 30, 100 ppm  
**Control Group:** yes  
**LOAEL:** 10 ppm

**Method:** other

**Method:** Groups of 5 male DBA/2J mice were exposed to 0, 10, 30 or 100 ppm (32, 96, 320 mg/m<sup>3</sup>) benzene (6 hr/d) for 5 days). One day and 5 days after the benzene exposures, the numbers of the two most primitive erythroid progenitor cells (BFU-E and CFU-E) and the numbers of the most primitive granulocytic progenitor cells (GM-CFU) were assessed.

**Result:** One day after benzene exposure, marrow erythroid progenitor cell numbers were depressed in all dose groups while marrow granulocytic progenitor cell numbers were unchanged or elevated. Five days after benzene exposure, the numbers of marrow erythroid progenitor cells have recovered from their depressed levels. In contrast, 5 days after exposure the numbers of granulocytic progenitor cells were depressed in the 10 ppm and 100 ppm groups. Normal ratio of granulocytic and erythroid progenitor cells were reported to be 17:1 in the marrow and 0.03:1 in the spleen reflecting the predominant erythroid hematopoiesis of the spleen. No clear treatment-related effects were seen for the splenic progenitor cells on day 1 after exposure, however on day 5 of recovery there is a dose-response type increase in splenic CFU-E numbers which was considered to possibly reflect the attempts by the spleen to repopulate the erythron after benzene exposure.

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(293)

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** 6 d  
**Frequency of treatment:** 6h/d  
**Doses:** 10, 31, 100 and 300 ppm (32, 99.2, 320, 960 mg/m<sup>3</sup> )  
**Control Group:** yes  
**LOAEL:** 10 ppm

**Method:** other  
**GLP:** no

**Result:** Inhalation exposure to dose levels of 0, 32, 99.2, 320, and 960 mg/m<sup>3</sup> (0, 10, 31, 100, and 300 ppm) benzene vapour to male C57B1/6J mice (5-7 males/group) on 6 days, 6 hr/day, produced a depression on lymphocyte counts at all dose levels and lower RBC counts at 320mg/m<sup>3</sup> (100 ppm) and above. Reduced numbers of B-lymphocytes (sIgM+ cells) in the femoral marrow and reduced T-lymphocytes in the spleen (Thyl.2+ cells) were observed at doses of 320mg/m<sup>3</sup> (100 ppm) and above.

Lymphoproliferative response to mitogens  
 Reduced RBC counts and numbers of T- and B-lymphocytes (see Section 4.1.2.6.1 A) were observed at dosages of 100 ppm (320 mg/m<sup>3</sup>) and above. Levels of circulating lymphocytes and lipopolysaccharide (LPS)-induced B-colony forming ability of femoral B-lymphocytes were depressed at all dose groups. At 31 ppm (99 mg/m<sup>3</sup>) splenic phytohemagglutinin (PHA)-induced blastogenesis of T-lymphocytes was also depressed.

**Source:** German Rapporteur

**Flag:** Risk Assessment

27-OCT-2000

(974)

**Species:** mouse **Sex:** male  
**Strain:** Balb/c  
**Route of administration:** inhalation  
**Exposure period:** 7 or 14 d  
**Frequency of treatment:** 6 h/d  
**Doses:** 50, 200 ppm  
**Control Group:** yes  
**LOAEL:** 50 ppm

**Method:** other

**Method:** BALB/c male mice (5-6 animals/group) were exposed to 50 or 200 ppm benzene vapour, 6 hr/d for 7 or 14 consecutive days.

Cell-mediated immune response was measured by contact sensitivity (CS) to picryl chloride (PCl) in BALB/c male mice exposed to 50 or 200 ppm benzene vapour (6 hr/d) for 14 consecutive days. Mice were immunized with PCl on day 7 or 9 and challenged to PCl on day 14. The CS response expressed as the increase of ear thickness after 6, 24, and 48 hr was enhanced in the 200 ppm group.

The activity of suppressor cells was evaluated in spleen by

the suppressive effect on splenic passive transfer of CS. Spleen cells from 14-days exposed mice which were immunized with PCl on day 7 and killed on day 14 were injected into irradiated recipients which received effector cells from immunized nonexposed animals. The response was measured as the increase of ear swelling at 24 h after challenge to PC of the recipients.

**Result:** No deaths or body weight suppression were observed. Relative spleen weight decreased in all treatment groups. Also, the relative thymus weight decreased in all groups except for the 50 ppm group treated on 7 days. WBC counts were depressed at 200 ppm at both treatment periods and at 50 ppm after 14-day exposure. RBC counts did not show treatment-related effects. The absolute numbers of B- and T-lymphocytes in blood and spleen were depressed related to the doses at 7 and 14 days of exposure in both dose groups. Spleen and blood percentages of B-lymphocytes were depressed in both dose groups. The depression was dose dependent. The T-lymphocyte ratio increased in the high dose group after 7 days of exposure and in both dose groups after 14 days of exposure.

#### Contact Sensitivity (CS)

It was concluded that the activity of T-lymphocytes in the induction and expression of CS was not depressed at the exposure levels.

#### Suppressor cell activity

Ear thickness increased at 200 ppm to comparable percentages of the positive controls assuming that suppressor cell activity in mice exposed to 200 ppm was significantly lowered.

#### Humoral immunity

Humoral immune response to sheep red blood cells (SRBC) was depressed in male BALB/c mice, which were exposed to 50 or 200 ppm of benzene vapour for 14 consecutive days showing reduced numbers of IgG- and IgM- plaque-forming cells per spleen in the plaque-forming cell assay (PFC).

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(35)

**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of administration:** inhalation  
**Exposure period:** 4-10 d  
**Frequency of treatment:** continuous (24h/d)  
**Doses:** 1, 10, 21, (61mg/m<sup>3</sup>), 50, 95 ppm  
**Control Group:** yes  
**NOAEL:** 10 ppm

**Method:** other

**Method:** Groups of 5 animals were exposed to 21 ppm (61 mg/m<sup>3</sup>), 50 and 95 ppm benzene for 4-10 days (24 hr/d). Parameters examined in bone marrow/tibia: cellularity, number of colony-forming unit granulopoietic stem cells (CFU-C) and micronucleated polychromatic erythrocytes (MN-PCE).

**Result:** NMRI mice at continuous showed reduction in bone marrow cellularity, number of colony-forming unit granulopoietic stem cells (CFU-C) and an increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCE). No effects were seen at 1 and 10 pp

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(1143)

**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of administration:** inhalation  
**Exposure period:** 1-8 wk  
**Frequency of treatment:** continuous (24 h/d)  
**Doses:** 14 ppm (45 mg/m<sup>3</sup>)  
**Control Group:** yes  
**LOAEL:** 14 ppm

**Method:** other

**Remark:** Parameters examined in bone marrow/tibia: cellularity, number of colony-forming unit granulopoietic stem cells (CFU-C) and micronucleated polychromatic erythrocytes (MN-PCE).

**Result:** Mice exposed continuously to 14 ppm (45 mg/m<sup>3</sup>) for 1-8 weeks had significant increased number of micronucleated polychromatic erythrocytes (MN-PCE).

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(1143)

**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of administration:** inhalation  
**Exposure period:** 2 wk  
**Frequency of treatment:** 8 hr/d, 5 d/wk or additional experiments on 0-8 hr/d, 5 d/wk  
**Doses:** 0, 10, 21, 50, 95, 107 ppm  
**NOAEL:** 10 ppm

**Method:** other

**Method:** Additional experiments included exposure to 95 and 201 ppm on 0-8 h/d, 5 d/w for 2 weeks. Parameters examined in bone marrow/tibia: cellularity, number of colony-forming unit granulopoietic stem cells (CFU-C) and micronucleated polychromatic erythrocytes (MN-PCE).

**Result:** Intermittent exposures on 8 hr/d, 5 d/w during 2 weeks to 10, 21, 50, 95, or 107 ppm revealed that exposure to 21 ppm was the lowest dose tested yielding suppressed CFU-C content and elevated frequency of MN-PCE. Bone marrow cellularity was depressed at doses of 50 ppm and above.

Additional experiments including exposure to 95 and 201 ppm on 0-8 h/d, 5 d/w for 2 weeks revealed depressive effects on bone marrow cellularity and CFU-C content at concentration of 95 ppm at the 6 hr/d regimen, the number of MN-PCE were increased at 4 h/d exposure time. 201 ppm for 2 hr/d suppressed cellularity and increased numbers of MN-PCE, but did not alter significantly CFU-C content indicating that at high exposures of short duration bone marrow cell numbers were the most sensitive parameter. At the 4 hr/d dose regimen 201 ppm caused changes of all three parameters.

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(1143)

**Species:** mouse **Sex:** male/female  
**Strain:** other: C57BL1/6 BNL  
**Route of administration:** inhalation  
**Exposure period:** 2 wk  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Doses:** 10, 25, 100, 400 ppm (32, 80, 320, 1280 mg/m<sup>3</sup>)  
**Control Group:** yes  
**NOAEL:** 10 ppm

**Method:** other

**Method:** Male and female C57B1/6 BNL mice (5-10 animals/group) were exposed to benzene vapour at concentration of 0, 10, 25, 100, or 400 ppm (32, 80, 320, or 1280 mg/m<sup>3</sup>) for 2 weeks (6 hr/d, 5 d/w).

**Result:** At 100 ppm reduced bone marrow cellularity (no data on higher doses) was reduced. A decreased number of pluripotent stem cells in bone marrow and a higher fraction of stem cells in DNA synthesis were reported at 100 and 400 ppm. In peripheral blood hematocrit was reduced at 100 ppm and above

and lymphocyte counts were depressed at 25 ppm and higher. No effect on the granulocytes was seen in all dose groups. Hemosiderin deposits in the spleen were reported in exposed animals, however no exact data are available.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(261)

**Species:** mouse **Sex:** male

**Strain:** other: C57B1/6J and Swiss Webster

**Route of administration:** inhalation

**Exposure period:** 2 wk

**Frequency of treatment:** 6 h/d, 4 d/wk

**Doses:** 300 ppm (960 mg/m<sup>3</sup>)

**Control Group:** yes

**LOAEL:** 300 ppm

**Method:** other

**Method:** Male mice of each strain were exposed to 300 ppm benzene (960 mg/m<sup>3</sup>) for 6 hours per day, 4 days per week, for 2 weeks.

**Result:** Indicating strain-specific differences in the response to benzene, susceptibility was higher in Swiss Webster mice than in C57B1/6J mice. They had reduced numbers of bone marrow cells and a reduced development of CFU-E (colony-forming unit-erythroid) with a more severe reduction in the Swiss Webster mice.

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(822)

**Species:** mouse **Sex:** male

**Strain:** other: C57B1/6

**Route of administration:** inhalation

**Exposure period:** up to 6 wk

**Frequency of treatment:** 6 h/d, 5 d/wk

**Doses:** 1000, 2000, 4000 ppm

**Control Group:** yes

**LOAEL:** 1000 ppm

**Method:** other

**Method:** 6 males/group

**Result:** Intermittent exposures of 6 males/group on 5 days per week, 6 hr/day for up to 6 weeks resulted in a concentration-related leukopenia at dosages of 1000, 2000, and 4000 ppm. Decreased numbers of white blood cells were evident on the third day and reached the minimal number by the 5th or 6th day of exposure. Mortality from continuing the treatment up to 6 weeks was negligible. Mice of the 4000 ppm group experienced tremulousness and were nearly immobilized during the 6-h exposure. Animals recovered quickly after the return to room air. Differential blood counts performed on the high dose animals after counts stabilized during treatment revealed decreased numbers of lymphocytes (1.34x versus 5.94x10<sup>3</sup>/cells/ $\mu$ l in controls) and

granulocytes (0.5x versus  $2.37 \times 10^3$ /cells/ $\mu$ l in controls). Cellularity of femoral marrow was not altered at 4000 ppm concentration. However, number of splenic colonies of transplantable colony forming units (CFU-S) representing marrow precursors of granulocytes was reduced to about 55% after 1- and 4-week exposure and to 30% after 6-week exposure.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
25-OCT-2000

(411)

**Species:** mouse **Sex:** male  
**Strain:** other: C57B1/6  
**Route of administration:** inhalation  
**Exposure period:** up to 8 days  
**Frequency of treatment:** continuous (24 h/d)  
**Doses:** 100, 500, 1000, 2000, 4000 ppm  
**Control Group:** no data specified  
**LOAEL:** 100 ppm

**Method:** other

**Result:** Continuous exposure of mice to concentrations of 4000 ppm and 2000 ppm resulted in death of the mice within 24 hours. Causes were reported to be unrelated to the hematopoietic system (no further data). Continuous exposures to 1000 and 500 ppm caused death after 3 or 4 days of exposure. Exposure to 100 ppm were tolerated for longer than 1 week. A decline of white blood cell numbers occurred in the 100, 500, and 1000 ppm groups beginning already after 24 hours. Bone marrow cellularity exposed continuously to benzene at concentrations of 500 and 1000 ppm was not altered during the first 24 h, but at the end of 48 h to about 30% of the control values.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
25-OCT-2000

(411)

**Species:** mouse **Sex:** male  
**Strain:** other: B6C3F1/Cr1BR  
**Route of administration:** inhalation  
**Exposure period:** 1,2,4,8 wk  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Post exposure period:** 4, 11, 18 and 25 d  
**Doses:** 1,5,10,100, 200 ppm  
**Control Group:** yes  
**NOAEL:** 10 ppm

**Method:** other

**Method:** Male B6C3F1/Cr1BR mice (24 animals/group) were exposed to 0, 1, 5, 10, 100, and 200 ppm benzene for 6 hr/d, 5 days/week for 1, 2, 4, or 8 weeks. A subset of mice from the 4-week exposure was kept for 4, 11, 18, and 25 days of recovery.

**Result:** There was no significant effect on hematopoietic parameters from exposure to 10 ppm or less. Exposure of mice to 100 or 200 ppm benzene reduced the number of total bone marrow cells at all time points. A tendency to recover was obvious at the 4th week of the treatment in the 100 ppm group, but the decrease progressed in the 200 ppm animals. Bone marrow nucleated cells were within control values by 4 days after the end of exposure to 100 and 200 ppm for 4 weeks. CFU-HPP representing highly proliferative potential primitive progenitor cells of all three lineages (comparable to CFU-S of other studies) were decreased at all time points in the 200 ppm group and at 2, 4, and 8 weeks in the 100 ppm group. The number remained decreased in the 200 ppm recovery group and returned to control values by day 11 in the 100 ppm recovery group. Replication of primitive progenitor cells, measured as the percentage of these cells in S-phase of the cell cyclus, increased during the exposure period as a compensation for the cytotoxicity induced by 100 and 200 ppm benzene. In mice exposed to 200 ppm benzene, the primitive progenitor cells maintained an increased percentage of cells in S-phase through 25 days of recovery compared to controls. At the 100 ppm recovery group, percentages of cells in S-phase remained high until day 11 postexposure. Exposure to 100 or 200 ppm benzene induced an increase in the number of erythrocytic bone marrow colony forming units (CFU-E) after 1 week of exposure and, at the high dose, a decrease after 2 and 8 weeks of exposure. While the absolute numbers of differentiating erythropoietic cells, characterized by immunostaining to be rubriblasts, rubricyts, and metarubricytes, were decreased at 100 and 200 ppm benzene, the percentage of immunolabelled cells increased by exposure to 100 ppm for 4 weeks and 200 ppm for 8 weeks. A decrease in the percentage of PCE in the blood was severe at 5 days of exposure to 100 or 200 ppm benzene. The absolute numbers and percentages of PCE were decreased in the high dose group through week 4 and in the 100 ppm group through week 2 (no data on week 1). Erythrocytes in the blood were diminished from the second week onwards in the 100 ppm group and at all time points in the 200 ppm group. The mean corpuscular volume increased at week 8 of treatment with 100 and 200 ppm. The number of granulocyte-macrophage colony-forming

units (CFU-GM) was reduced by 100 and 200 ppm benzene with exposure for 2, 4, or 8 weeks. The absolute numbers of granulocytic marrow cells recognized by immunolabelling and morphologically classified to be myeloblasts, promyelocytes, myelocytes, band cells, and segment neutrophils, were decreased by 100 ppm benzene at week 4 and 200 ppm benzene at all time points, whereas the percentages of these cells increased by exposure to 100 or 200 ppm benzene for 2 weeks and by exposure to 100 ppm for 8 weeks. In mice recovering from 200 ppm benzene, the number of granulocytic cells increased above controls at 4 days postexposure and thereafter returned to the control values. The numbers of blood leukocytes were lowered at from week 2 onwards (no data on week 1) in mice exposed to 100 and 200 ppm benzene. The numbers of platelets were reduced in mice exposed to 200 ppm from week 2 through week 8 and in mice exposed to 100 ppm at week 2.

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(348)

**Species:** mouse

**Sex:** male

**Strain:** CD-1

**Route of administration:** inhalation

**Exposure period:** Experiment (exp.) 1: 5 d; exp. 2: 50 d; exp. 3: 26 wk

**Frequency of treatment:** 6 h/d, 5 d/wk

**Doses:** exp.1: 1.1,10,100, 306,603,1276,2416,4862 ppm; exp. 2: 9.6 ppm; exp.3: 302 ppm

**Method:** Green et al. (1981a,b) conducted 3 experiments using different exposure regimens and concentrations to evaluate the interaction of inhaled benzene with hematopoietic stem cells (multipotential hematopoietic stem cell CFU-S, granulocyte/macrophage progenitor cell CFU-GM), marrow and spleen cells. In experiment 1, male CD-1 mice (11-19 animals/dose group) were exposed for 6 hr/d for 5 days to 3.5, 32, 320, 979, 1930, 4083, 7731, or 15558 mg/m<sup>3</sup> (1.1, 10, 100, 306, 603, 1276, 2416, or 4862 ppm). Experiment 2 was designed to compare the effects of 32 mg/m<sup>3</sup> (10 ppm) exposure delivered over 50 days (6 hr/d, 5 d/w) to effects from 320 mg/m<sup>3</sup> (100 ppm) exposure delivered over 5 days (experiment 1). In experiment 3, mice were exposed to 966 mg/m<sup>3</sup> (302 ppm) for 6 hr/d, 5 d/w for 26 weeks. There were no exact data on the number of animals/group in experiments 2 and 3, number of animals for organ cellularity and weight determination was 12 males/group.

**Result:** Results from experiment 1 showed that spleen weight, femoral and splenic cellularities (total number of nucleated cells, granulocytes, lymphocytes and nucleated red cells), total number of CFU-S in femur and spleen, and the number and concentration of splenic CFU-GM were significantly reduced at concentrations <sup>3</sup> 320 mg/m<sup>3</sup> (100 ppm). In femur, absolute numbers of CFU-GM were marginally reduced at 100 ppm and significantly lower at all higher doses, whereas the fraction of CFU-GM was increased to variable amount in most doses of 100 ppm and higher. Exposure to 306 ppm resulted in

reduced concentration of splenic and marrow CFU-S. In peripheral blood, WBC, neutrophils and lymphocytes were depressed  $\approx 320 \text{ mg/m}^3$  (100 ppm). RBC counts were depressed only at the two highest exposure levels.

Experiment 2 showed that exposure to  $32 \text{ mg/m}^3$  (10 ppm) over 50 days resulted in higher spleen weight, elevated splenic cellularity and increased number and concentration of CFU-S, but no changes in the CFU-S content of bone marrow were detected. CFU-GM were not evaluated in this experiment. No differences in the peripheral blood, bone marrow, or body weight were detected in exposed mice.

Results from experiment 3 showed lower spleen weight, marked depression in marrow and spleen cellularity with depressed marrow and spleen CFU-S (total number and concentration) and marrow CFU-Gm (total number and concentration) and spleen CFU-GM (total number). Marked changes in the peripheral blood included depressed WBC counts, RBC counts and percentages of lymphocytes, while the number of neutrophils appeared to be elevated. Morphologically neutrophils were abnormal exhibiting pyknosis and hypersegmentation. Red cell morphology was characterized by polychromasia, anisocytosis, poikilocytosis, stippling, and numerous Howell-Jolly bodies. Marrow differentials revealed reduced numbers of granulocytes, lymphocytes and nucleated red cells. In spleen number of lymphocytes were more drastic reduced than granulocytes, while the number of nucleated red cells were equal to the control value. Morphologically, nucleated marrow and spleen cells displayed a variety of nuclear/cytoplasmic dyscrasias including nuclear and cytoplasmic blebbing, vacuolization, and atypical mitotic figures. In addition, asynchronous nuclear/cytoplasmic maturation was observed in myeloid precursors.

**Source:** German Rapporteur  
25-OCT-2000

(441)

**Species:** mouse **Sex:** male  
**Strain:** other: Hale Stoner BNL  
**Route of administration:** inhalation  
**Exposure period:** up to 65 d  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Post exposure period:** 14 d  
**Doses:** 400 ppm  
**LOAEL:** 400 ppm

**Method:** other

**Method:** Effects of benzene inhalation on mouse pluripotent hematopoietic stem cells have also been evaluated in the study of Cronkite and coworkers (1982). Male Hale Stoner BNL mice were exposed to 400 ppm benzene for 6 hr/d, 5 d/w, for up to 9½ weeks (65 days) with a 14 day-recovery period. At various times during and after the exposure period two to four mice were sacrificed to examine WBC and RBC counts, femur and tibia were evaluated for total bone marrow cellularity, stem cell content and the percent of stem cells

in DNA synthesis.

**Result:**

In extended experiments of this study, assays of early progenitors of erythrocytic cells and granulocytic cells were performed (Cronkite et al. 1989). Exposure to benzene caused depressions of RBC and WBC counts gaining significance on day 11 and day 4 of exposure, which continued throughout the study and for at least 14 d after exposure. Beginning at the 5th day of exposure bone marrow cellularity were also depressed in exposed animals throughout the study. Except the day 50 cellularity was decreased to 33% up to the 25th day of study and around 50% for the remaining time to the control values. After the termination of benzene exposure, the marrow cellularity increased promptly to 88% of the control values 14 days after the last exposure. The absolute stem cell content measured as colony-forming unit in spleen (CFU-S) had dropped to 23% of the control value at day 5 of exposure and remained between 13 and 43% up to 14 day after termination of exposure. This effect was explained by a large reduction in the amplifying populations of indentifiable erythrocytic and granulocytic precursors. An initial decrease of CFU-S actively synthesizing DNA from 26 to 13% during the first 3 days of exposure was evident, thereafter the percentage increased ranging from 26% to 66% during the remaining time of exposure and dropped after the last exposure to 7%. 5-25% of the CFU-S of control animals were in DNA synthesis. Histological typing of splenic colonies produced by bone marrow from exposed mice revealed that immature colonies have disappeared on the day 3 of exposure followed by a short rebound and a secondary diminution. Mature erythrocytic and granulocytic colonies diminished more slowly, reaching a minimum on day 5 and remaining lower than control levels throughout the study.

In extended experiments of this study, assays of early progenitors of erythrocytic cells and granulocytic cells were performed (Cronkite et al. 1989). 2-day cultures of erythrocyte colony forming units (CFU-E) from bone marrow cell suspensions were not changed after one and four exposures, but after 29, 48 and 65 days of exposure there was a significant diminution in the number of CFU-E. Bone marrow derived eight-day burst forming units (BFU-E-8) cultured in erythropoitin and pokeweed-mitogen were reduced markedly by day 29 of exposure (<10% of control levels) and recovery was incomplete 12 days after termination of exposure. Units of granulocyte-macrophage aggregates in agrar cultures were decreased compared to controls. The decrease was smaller (<20%) than that of BFU-E-8 with recovery to nearly that of the control levels 12 days after termination of exposure.

**Source:**

German Rapporteur

**Flag:**

Risk Assessment

06-JUL-2005

(262) (263)

**Species:** mouse **Sex:** male/female  
**Strain:** other: C57B1/6BNL  
**Route of administration:** inhalation  
**Exposure period:** 2,4,8,16 wk  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Doses:** 10, 25, 100, 300, 400 ppm (converts to 0.03, 0.08, 0.33, 0.98, 1.3 mg/l);  
**Control Group:** yes  
**NOAEL:** 10 ppm

**Method:** other

**Remark:** Control group [numbers unspecified] were exposed to conditioned air. At various times during and after the exposure period, five to ten mice were removed from both the treated and control groups and the blood, bone marrow and spleens removed and examined.

**Result:** Rapporteur: Exposure to 300 ppm (960 mg/m<sup>3</sup>) for 2, 4, 8, and 16 weeks produced reduced lymphocyte counts and a lower level of pluripotent stem cells in bone marrow of male and female C57B1/6 BNL mice which returned to those of controls 2 to 4 weeks after benzene exposure for 2 and 4 weeks, 16 weeks after exposure for 8 weeks, and to 92% of controls 25 weeks after 16 weeks of exposure. There was a more rapid return of blood lymphocytes to control level. A significant difference of lymphocyte counts was observed at 2 and 4 weeks postexposure after 4 weeks of exposure to 300 ppm. There was no difference at 8 and 16 weeks postexposure. Similar regression courses was seen for other exposure regimens. Mice exposed to 300 ppm for 16 weeks had a shorter latency period of mortalities than control animals.

IND: The lowest benzene concentration caused a slight decrease in the fraction of haemopoietic stem cells in DNA synthesis after 2 wk exposure. This, however, was only determined once at this level and no such effect was seen at 0.08 mg/l. A dose-related effect on diminution of lymphocyte concentration in the peripheral blood was apparent at 0.08 mg/l or more after 10 exposures in 12 days. Exposures for this same time to concentrations of 0.33 mg/l and above significantly depressed the femur bone marrow cellularity, the CFU levels in the femur and the haematocrit and caused a slight increase in fraction of haemopoietic stem cells in DNA synthesis. This last finding is of questionable significance. The depressed CFU and blood lymphocyte levels seen following exposure to 0.98 mg/l for 2, 4, 8 and 16 weeks had almost completely recovered 16 weeks after ceasing exposure. Early mortality and an increase in mortality rate were noted after 16 weeks exposure to 0.98 mg/l.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(261)

**Species:** mouse **Sex:** female  
**Strain:** other: BDF1  
**Route of administration:** inhalation  
**Exposure period:** 16 wk  
**Frequency of treatment:** 6 h/d, 5 d/w  
**Post exposure period:** recovery up to 185 d  
**Doses:** 100, 300, 900 ppm  
**Control Group:** yes  
**LOAEL:** 100 ppm

**Method:** other

**Method:** Bone marrow hemopoietic stem cell compartments and peripheral blood cell counts were studied in female BDF1 mice (9 animals/group) exposed for 16 weeks to 100, 300, and 900 ppm of benzene, 6 hours per day, 5 days per week.

**Result:** Dose-dependent depressive effects were observed on all stem cell compartments. Only the erythroid colony-forming units (CFU-E) compartment was depressed during exposures to 100 ppm. CFU-E were more sensitive than the erythroid burst-forming units (BFU-E), spleen CFU (CFU-S), or CFU-GM, which were depressed by exposure to 300 ppm or 900 ppm. In peripheral blood, lymphocytopenia developed in mice exposed to 300 and 900 ppm benzene. At these dose groups, erythrocyte counts were depressed at week 4, did not further progress and showed a tendency to recover at week 13 and 16. After benzene-free intervals, a regeneration of lymphocyte numbers and slow normalisation of stem cell numbers was seen. Complete recovery from the 16 weeks exposure to 300 ppm was seen between 73 and 185 days.

**Source:** German Rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(1027)

**Species:** mouse **Sex:** male/female  
**Strain:** other: CBA/Ca BNL  
**Route of administration:** inhalation  
**Exposure period:** exp.1: 19 exposure; exp. 2: 2 exposures  
**Frequency of treatment:** 6 h/d; 5 d/wk  
**Post exposure period:** up to 214 d  
**Doses:** exp. 1: 316 ppm (1011 mg/m<sup>3</sup>); exp. 2: 3000 ppm (9600 mg/m<sup>3</sup>)  
**Control Group:** yes  
**LOAEL:** 316 ppm

**Method:** other

**Method:** To compare benzene effects of short term exposure with high dosages with longer exposure regimen at lower doses, Cronkite et al. (1989) exposed male and female CBA/Ca BNL mice (no data on number of animals/group) to 316 ppm (1011 mg/m<sup>3</sup>), of benzene vapour on 6 hr/d, 5 d/w, for a total of 19 exposures. Another group received 3000 ppm (9600 mg/m<sup>3</sup>), 6 hr/d, for two successive exposures.

**Result:** After termination of exposure and up to 214 days after termination of exposure, lymphocyte and neutrophil counts were reduced in either treatment group. However, lymphopenia

and neutropenia were more drastic in the longer exposure regimen at most periods of recovery. At day 214 after exposure, only neutrophilic counts had recovered in the 3000 ppm group. Similarly differential leukocyte counts demonstrated reduction of all cell types except large unstained cells. Bone marrow cellularity was reduced in both treatment groups on day 1 after treatment and recovered at day 32. The content of marrow colony forming unit-spleen (CFU-S) were reduced in both treatment groups at day 1 of recovery. Whereas this parameter remained lowered up to 214 days in the 316 ppm group, it recovered by day 32 in the 3000 ppm group.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
25-OCT-2000

(262)

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** up to 178 days  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** none  
**Doses:** 10 ppm (converts to 0.032 mg/l); group numbers unspecified  
**Control Group:** yes, concurrent no treatment  
**LOAEL:** 10 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Method:** C57B1/6J male mice were exposed to 32 mg/m<sup>3</sup> (10 ppm) benzene for 6 hr/day, 5 days/week for up to 178 days (no number on animals tested). In vivo and in vitro evaluations of hematopoiesis, specifically erythropoiesis, were performed at 32, 66 and 178 days of exposure.

**Remark:** Chamber atmospheres were analysed at half-hour intervals during the daily 6-hour exposures. Control mice received filtered conditioned air. After 32, 66 and 178 days, mice from each group were bled and the peripheral blood cell counts determined. Five animals from each group were selected for red progenitor cell and nucleated red cell determinations.

**Result:** There were significant depressions in the numbers of circulating RBC and lymphocytes in benzene-exposed mice. The levels of circulating neutrophils, however, were unaffected by the exposures (data not shown). At 178 days, benzene-exposed mice exhibited depressions in splenic nucleated cellularity and in splenic nucleated RBC numbers. Marrow cellularity and marrow-nucleated RBC counts were unaffected by the exposures (data not shown). In vitro, progenitor cells from benzene-exposed mice showed reduced ability to form colonies compared to cells from control mice. Benzene exposed mice showed a progressive decline in bone marrow and splenic colony-forming unit-erythroid (CFU-E) colonies during the exposure period, reaching 5% and 10%, respectively, of control values after

178 days.

Marrow burst-forming unit-erythroid (BFU-E) colonies were significantly depressed at day 66 but had returned to normal by day 178. No effect was seen at any monitoring period on splenic BFU-E colony growth.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(64)

**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of administration:** inhalation  
**Exposure period:** 13 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** none  
**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 100 mice/sex/dose  
**Control Group:** yes, concurrent vehicle  
**NOAEL:** 1 ppm

**Method:** other: in compliance with OECD Guide-line 413

**Year:** 1981

**GLP:** no data

**Test substance:** other TS

**Remark:** In this study, 150 mice were exposed to benzene vapour (whole body exposure) at concentrations of 3.2, 32, 96 or 960 mg/ m<sup>3</sup> (1, 10, 30 or 300 ppm) for 6 hr/day, 5 days/week for up to 13 weeks, 20 mice/sex/group sacrificed after 7, 14, 28, 56, and 91 days of treatment (study design in accordance to the requirements of 412).

All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained at necropsy from 20 randomly selected mice/sex/group for clinical pathology (hematology and clinical biochemistry). Complete necropsies, organ weight analysis and histopathology were performed on 20 of these sacrificed animals/sex and on all animals found dead or moribund.

**Result:** Exposure to benzene showed decreased WBC counts in CD-1 mice exposed to 960 mg/m<sup>3</sup> (300 ppm) for 2 to 13 weeks. No exposure-related mortality or effects on mean body weight and clinical signs were seen. At 960 mg/ m<sup>3</sup> (300 ppm), mice exhibited statistically significant decreases in hematocrit, Hb, RBC count, WBC count, platelet count, myeloid/erythroid ratio and the percentage of lymphocytes at day 14 of treatment and later. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and the incidence and severity of red cell morphologic cell changes were increased in mice. These changes included anisocytosis, poikilocytosis, acanthocytosis, hypochromasia, nucleated red blood cells, Howell-Jolly bodies, polychromasia, echinocytosis and

basophilic stippling. Gross pathology observations in mice included a slight increase in the incidence of small thymuses (day 56 and 91) and small spleens (day 56) in the 300 ppm group. At several sacrifices, animal of this dose groups showed lower organ weights in the testes, female mice had higher liver weights. Histopathology changes in 300 ppm mice of all sacrifice groups were first seen at day 7 of treatment and increased in severity with time. They included myeloid hypoplasia of the femoral marrow, depletion of the periarteriolar lymphoid sheaths in the spleen, lymphoid depletion in the mesenteric lymph nodes, and increased extramedullary hematopoiesis in the spleen. Plasma cell infiltration in the mandibular lymph nodes occurred in some mice at day 28 and thereafter. Additional, the final sacrifice revealed centrilobular hypertrophy of hepatocytes (three males). Degenerative lesions of the testes and the ovaries occurred in mice of the 300 ppm group as well as at lower dose groups. The NOAEL for hematological effects on peripheral blood circulation in this study was 96 mg/ m<sup>3</sup> (30 ppm). The NOAEL for all adverse effects was not clearly estimated. Lesions resembling those seen in the high dose mice were thymic atrophy, increased extramedullary hematopoiesis, plasma cell infiltration in mandibular lymph nodes in some animals of the 10 and 30 ppm dose groups.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.9% pure.  
**Flag:** Risk Assessment

06-JUL-2005

(1237)

**Species:** mouse **Sex:** male/female  
**Strain:** DBA  
**Route of administration:** inhalation  
**Exposure period:** 13 wk  
**Frequency of treatment:** 5 d/w or 3 d/w, both 6 h/d  
**Doses:** 300 ppm (960 mg/m<sup>3</sup>)  
**Control Group:** yes  
**LOAEL:** 300 ppm

**Method:** other

**Method:** No other test parameters than percentage of polychromatic erythrocytes (PCE), frequency of micronuclei in polychromatic erythrocytes (MN-PCE) and normochromatic erythrocytes (MN-NCE) in the peripheral blood.

**Result:** Frequency of MN-PCE (lifetime of PCE in mice: 24 h) was increased without regimen and exposure duration relationship. Exposure to benzene resulted in an duration-dependent increase of MN-NCE (lifetime in mice of around 30 days). The increase was more slowly in the exposure regimen on 3 consecutive days. Males were more sensitive than females to both effects. An analysis of % PCE data (3% in controls) revealed an initial severe depression in the rate of erythropoiesis in both sexes (almost 0%), with a return of the PCE production to control levels being dependent to both sexes and exposure regimen. The effect was

more persistent in males, exposure on 3 consecutive days were more depressive than on 5 days (only in males). However, during the later weeks of exposure there was a high variability of frequencies among individual control and exposed animals.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
06-JUL-2005

(708)

**Species:** mouse **Sex:** male  
**Strain:** other: DBA/2, B6C3F1, C57B1/6  
**Route of administration:** inhalation  
**Exposure period:** 13 wk  
**Frequency of treatment:** 5 d/w or 3 d/w, both 6 h/d  
**Doses:** 300 ppm (960 mg/m<sup>3</sup>)  
**LOAEL:** 300 ppm

**Method:** other

**Method:** similar to Luke et al., 1988a

**Result:** The frequency of MN-PCE increased in all strains. The magnitude was strain specific (DBA/2 > C57B1/6 = B6C3F1), independent from exposure regimen and, except for exposure on 3 consecutive days in B6C3F1 mice, of exposure duration. There was an exposure duration-dependent increase in the frequency of MN-NCE in exposed mice of all strains (5 exposure days > 3 exposure days). The magnitude of MN-NCE accumulation were different between the strains, however differences were inhomogeneous between treatment regimen (5 exposure days: C57B1/6 = B6C3F1 > DBA/2, 3 exposure days: C57B1/6 > B6C3F1 = DBA/2). PCE levels were markedly depressed in the peripheral blood of mice of all strains. The extent and duration was dependent on both strain (more pronounced in DBA/2 mice) and exposure regimen (3 exposure days > 5 exposure days). The group mean percentage of PCE varied throughout the course of the study, variability was even greater within individuals from one week to another particularly evident near the final weeks of the study.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
25-OCT-2000

(709)

**Species:** mouse **Sex:** male  
**Strain:** other: C57B1/6J  
**Route of administration:** inhalation  
**Exposure period:** 6 consecutive days or 30 and 115 exposure days (5 d/w)  
**Frequency of treatment:** 6 h/d  
**Doses:** 300 ppm  
**Control Group:** yes  
**LOAEL:** 300 ppm

**Method:** other

**Method:** Groups of ten C57B1/6J male mice were exposed to 300 ppm via inhalation for 6 exposures on 6 consecutive days (6 hr/d) or for 30, and 115 exposures (6 hr/d, 5 d/w). Histopathology was not performed

**Result:** In peripheral blood, total lymphocytes and RBC were depressed after 6, 30, and 115 exposures. Depressions in mean counts of lymphocytes and RBC intensified with exposure progression. Mean spleen and thymus weights were also lower after all exposure periods compared to air-exposed controls. Thus, spleen and thymus weight of benzene-exposed mice increased with time. Nucleated cells in spleen, bone marrow and thymus from benzene-exposed mice were depressed after all periods of exposure. However, exposed mice exhibited a 15-fold increase in thymic cellularity and a 3-fold increase in marrow cellularity between 6 and 30 exposures. The total number of B-lymphocytes in bone marrow and spleen and the numbers of T-lymphocytes in thymus and spleen were found to be markedly reduced after all three periods. The numbers of splenic B-lymphocytes were continuously declining during exposure reaching less than 1% of control values, the marrow B-lymphocyte numbers were depressed to 6%, 11%, and 28% of corresponding air controls during the exposure periods. Similarly, the splenic T-lymphocytes were reduced progressively, the number of T-lymphocytes in thymus increased 15-fold in the period between 6 and 30 exposures.

Although depressed in comparison to control values, the relative increase of lymphocytes with exposure duration may reflect the efforts to repopulate the bone marrow and the thymus. No comparable increases were observed in the spleen. Other than the bone marrow and thymus, the spleen, however, has no B-cell or T-cell restorative capacities. The authors did not explain the underlying mechanism (e.g. extramedullary hematopoiesis, histiocytosis or hemosiderosis) of spleen weight increase.

Lymphoproliferative response to mitogens  
Inhalative exposure to vapor concentrations of benzene of 300 ppm (960 mg/m<sup>3</sup>) for periods of 6, 30, or 115 days resulted in decreased number and proliferative capacity of T- and B-lymphocytes (Rozen and Snyder 1985) in male C57B1/6J mice. Mitogen-induced proliferation in a B-lymphocyte colony forming assay to bone marrow and splenic B-lymphocytes exhibited a progressive depression throughout the exposure period reaching a point of no observable mitogen-induced response after 115 exposures. Splenic T-cell

mitogen-induced proliferation in the PHA-stimulation index assay was also markedly depressed throughout the exposures, but there was no evidence of a progressive decline in this response during the exposures.

**Source:** German Rapporteur

**Flag:** Risk Assessment

27-OCT-2000

(973)

**Species:** mouse

**Sex:** male

**Strain:** other: AKR/J and C57B1/6J

**Route of administration:** inhalation

**Exposure period:** lifetime exposure

**Frequency of treatment:** 6 h/d, 5 d/wk

**Doses:** 100 ppm (320 mg/m<sup>3</sup>) for AKR mice, 300 ppm for C57B1 mice

**Control Group:** yes

**LOAEL:** 100 ppm

**Method:** other

**Method:** 50 AKR/J mice and 40 C57B1/6J mice/group

**Result:** Chronic exposure to benzene caused persistent lymphopenia and depressed RBC counts beginning after 1 exposure week in exposed male AKR/J mice and C57B1/6J mice. AKR mice transiently showed a tendency toward neutrophilia whereas in C57B1 mice neutrophilia was evident after 17 weeks and persisted until the end of study. Morphologic changes of RBC were observed in C57B1 mice consisting in anisocytosis and poikilocytosis which began after 4, respectively 15 weeks of exposure. Hyperlobulated, mature neutrophils were observed and a neutrophilic left shift with increased appearance of metapyelocytes, myelocytes, promyelocytes, and giant platelets were evident concurrently with the neutrophilia. Where 20% of the exposed AKR mice developed bone marrow hypoplasia and no increased neoplasm rate, 33% of C57B1/6J mice developed bone marrow hyperplasia limited to granulopoietic elements and increased rate of hematopoietic neoplasms.

**Source:** German Rapporteur

**Flag:** Risk Assessment

26-OCT-2000

(1070)

**Species:** mouse **Sex:** male  
**Strain:** other: C57Bl and CD-1  
**Route of administration:** inhalation  
**Exposure period:** 1 wk interrupted by 2 wk of nonexposure for lifetime or 10 wk continuously  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Post exposure period:** for 10 wk exposure: observation until death  
**Doses:** 300 ppm for interrupted exposure regimen and 1200 ppm for continuous exposure  
**Control Group:** yes

**Method:** other

**Method:** Groups of males C57Bl and CD-1 mice were exposed to benzene vapour using two different exposure protocols. One protocol consisted of repetitive weeklong exposures of 60 mice/strain to 300 ppm benzene, 6 hr/d, 5 d/w interrupted by 2 weeks of non-exposure until death. The second protocol consisted of exposures of 80 mice/strain to 1200 ppm benzene, 6 hr/d, 5d/w, for 10 weeks. After termination animals were allowed to live out their lives.

**Remark:** LOAEC 300 ppm and 1200 ppm, resp.

**Result:** Hematotoxicity in long term studies were also investigated by another study of Snyder and coworkers (1988). This regimen produced peripheral blood lymphocytopenia and a mild anemia during the 10 weeks of exposure, but after cessation of exposures, blood counts returned to control values. The 300 ppm benzene exposures induced lymphocytopenia and anemia throughout the study. (The exact tumor data of both studies are cited in section 4.1.2.8).

**Source:** German Rapporteur

**Flag:** Risk Assessment

26-OCT-2000

(1071)

**Species:** rat **Sex:** female  
**Strain:** Wistar  
**Route of administration:** inhalation  
**Exposure period:** 7 or 14 days  
**Frequency of treatment:** 8 hours/day  
**Post exposure period:** none  
**Doses:** 20, 50, 100, 300, 1000, 3000 ppm (converts to 0.065, 0.16, 0.33, 0.98, 3.26, 9.78 mg/l); 5-7 rats/dose  
**Control Group:** yes, concurrent no treatment  
**NOAEL:** 50 ppm

**Method:** other  
**GLP:** no data

**Method:** A short-term study focussed on leucocytic alkaline phosphatase (LAP) activity and leukocyte counts in female Wistar rats (5-6 animals/group) exposed to 20, 50, 100, and 300 ppm, 8 hr/d, for 7 days (Li et al. 1986). Additional groups were exposed to 1000 ppm and 3000 ppm for 7 or 14 days.

**Result:** Blood sampled from all animals. TS analysed by GC. Exposure to 100 and 300 ppm resulted in a dose-dependent

increase in LAP; leukocyte counts and body weight were reduced. Exposure at higher concentrations at 1000 ppm and 3000 ppm for 7 or 14 days did not cause any additional elevation in LAP. An additional group of rats exposed to 300 ppm benzene vapour confirmed the findings in LAP, serum alkaline phosphatase remained unchanged.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >=99% pure.  
**Flag:** Risk Assessment

06-JUL-2005

(686)

**Species:** rat **Sex:** male/female  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure period:** 20 wk  
**Frequency of treatment:** 4 h/d, 6 d/wk  
**Doses:** 14.6 mg/l  
**Control Group:** yes  
**LOAEL:** 14.6 mg/l

**Method:** other

**Method:** 20-week study on male and female rats (6 animals/group, no data on the strain) exposed to 14.6 mg/l benzene (4 hours/day, 6 days/week)

**Result:** Leukocyte alkaline phosphatase activity was increased and WBC counts were decreased (no exact data on WBC counts were reported).

**Source:** German Rapporteur  
**Flag:** Risk Assessment

26-OCT-2000

(1080)

**Species:** rat **Sex:** male  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** 2 wk or 4 wk  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Doses:** 30, 200, 400 ppm  
**Control Group:** yes  
**NOAEL:** 200 ppm

**Method:** other

**Method:** Exposure of benzene vapour at concentrations of 0, 30, 200, or 400 ppm (0, 32, 96, 640, 1280 mg/m<sup>3</sup>) for 6 hr/d, 5 days/week for 2 weeks (8 males/group) or 4 weeks (8 males/group) on Sprague-Dawley rats

**Result:** The number of splenic B-lymphocytes and the absolute spleen weight were significantly reduced after 2 weeks at 400 ppm. Non-significant dose-related lower spleen weight were observed in the low and mid dose groups after 2 weeks and in all treatment groups after 4 weeks. Lowered spleen cellularity was observed in all treatment groups at week 4 of treatment showing significance only at the high dose level. After 4 weeks of 400 ppm, there was a significant

reduction in absolute and relative thymus weight and spleen B- and T-lymphocytes. There was no effect on the bone marrow cellularity at any time or treatment group.

In male Sprague-Dawley rats, no significant effect on the humoral immune response was measured in an ELISA of serum anit-SRBC IgM. SRBC was injected four days prior to the completion of 2 or 4-week exposure to 30, 200, or 400 ppm benzene vapour (6 hr/d, 5 d/w).

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(952)

**Species:** rat

**Sex:** male/female

**Strain:** Sprague-Dawley

**Route of administration:** inhalation

**Exposure period:** 13 weeks

**Frequency of treatment:** 6 hours per day/5 days per week

**Post exposure period:** none

**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose

**Control Group:** yes, concurrent no treatment

**NOAEL:** = 10 ppm

**LOAEL:** = 30 ppm

**Method:** Directive 87/302/EEC, part B, p. 20 "Sub-chronic inhalation toxicity study: 90-day repeated dose study using rodent species"

**GLP:** no data

**Method:** 50 rats/sex/group were exposed to benzene vapour (whole body exposure) at concentrations of 3.2, 32, 96 or 960 mg/m<sup>3</sup> (1, 10, 30 or 300 ppm) for 6 hr/day, 5 days/week for up to 13 weeks, and 10 rats/sex/group sacrificed after 7, 14, 28, 56, and 91 days of treatment.

All animals were examined twice daily for mortality and moribundity throughout the study. Observations for signs of toxicity were made weekly. On days 7, 14, 28, 56 and 91, blood samples were obtained at necropsy from ten randomly selected rats/sex/group. Complete necropsies were performed on all of these sacrificed animals and on all animals found dead or moribund.

**Result:** No exposure-related mortality, clinical observations or mean body weight changes were seen. In the high-dosed animals, a significant decrease in the white blood cell count and, from day 14, the percentage of lymphocytes was noted. No exposure-related gross pathologic observations were reported. Females exposed to 0.098 mg/l had significantly increased mean thyroid weights and, on histopathological examination of the high-dosed animals, lower femoral bone marrow cellularity was noted on days 7, 28, 56 and 91. No data on morphology or cellularity are reported for the lower dosed animals.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.9% pure.  
**Flag:** Risk Assessment  
06-JUL-2005 (1237)

**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** 6-31 wk  
**Frequency of treatment:** 5 h/d, 4 d/wk  
**Doses:** several dosing regimens (15-831 ppm)  
**Control Group:** yes  
**LOAEL:** 15 ppm

**Method:** other

**Method:** 5 hr/d, 4 d/w:  
46 d/831 ppm  
39 d/65 ppm  
154 d/15 ppm

7 hr/d, 4 d/w:  
245 d/47 ppm  
54 d/44 ppm  
126 d/31 ppm  
88 d/29 ppm

**Result:** In an early less documented study of Deichmann et al. (1963) groups of 40 Sprague-Dawley rats were exposed for 5 hr/d on 4 d/w for 6 to a maximum of 31 weeks to benzene vapour (whole body exposure). Hematology was done at weekly or biweekly intervals in 10 male and 10 female randomly picked animals. Rats exposed to a mean concentration of 831 ppm on 32 days over a period of 46 days had reduced number of WBC in males and females after one week of exposure and thereafter. Another treatment with mean exposure to 65 ppm on 26 days over a total period of 39 days showed reduced number of WBC beginning at the treatment week 2 in females and week 4 in males. Leucopenia became also apparent in rats exposed to 47 ppm of benzene for 7 hr/d on 180 days over a total of 245 days. A mean benzene vapour concentration of 44 ppm induced leucopenia gaining significance in males at week 7 and in females at week 5. Animals were sacrificed at week 8 after 45-54 periods of exposure. Concentrations of 31 ppm on 126 days (7 hr/d, 4 d/w), 29 ppm on 88 days (7 hr/d, 4 d/w) or 15 ppm on 154 days (5 d/w) did not induce changes in WBC counts. The spleen of rats of the 15 ppm, 31 ppm and 47 ppm groups examined microscopically revealed a higher incidence and severity of hemosiderosis (females>males). The authors summarized that the degree of leucopenia was similar in rats exposed to 65 ppm or 831 ppm, but exposure to higher concentration induced an earlier response. The leucopenia was less severe in rats exposed to 44 and 47 ppm. Female rats were more susceptible to leucopenia than males. The NOAEL for effects on peripheral blood was 31 ppm. However, no clear NOAEL for all adverse effects was established due to increased incidence/severity of splenic hemosiderosis at doses 15 ppm and above.

**Source:** German Rapporteur

**Flag:** Risk Assessment (289)  
26-OCT-2000

**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of administration:** drinking water  
**Exposure period:** 28 days  
**Frequency of treatment:** continuous  
**Post exposure period:** none  
**Doses:** 31, 166, 790 mg/l (estimated average doses of 8, 40, 180 mg/kg bw/day); numbers of animals unspecified  
**Control Group:** yes, concurrent no treatment  
**LOAEL:** 8 mg/kg

**Method:** other  
**GLP:** no data

**Method:** Adult CD-1 mice (5 males/group) administered orally via drinking water to 8, 40, or 180 mg/kg bw/d benzene for 4 weeks.

Gross and pathological examinations performed on all mice; major organs were removed and weighed; blood examined. Actual concentrations of benzene in the drinking water were determined by gas chromatographic analysis on several days and were found to range from 28 to 36, 147 to 178 and 734 to 855 mg/l for the three levels respectively. Based on the observed average intakes, the daily dose of benzene in each animal was estimated to be 8, 40 and 180 mg/kg bw respectively.

**Result:** No overt clinical symptoms of toxicity, no change in food and water consumption, no gross lesions on any of the organs, and no apparent alteration in body weight gain were seen, although the mid- and high-dose groups had slight non-significant increases in growth rate.

Peripheral RBC, blood leukocyte and lymphocyte counts and hematocrit were significantly reduced in a dose-responsive fashion, the latter being statistically significant only in the mid- and high-dose levels, while mean corpuscular volumes increased significantly as the dose increased. Numbers of neutrophils and other WBC were not altered. Benzene produced a dose-related decrease in spleen weight and increase in kidney weight at all dose groups gaining significance at the high dose level. Thymus weight was reduced at all dose groups but not significantly. The total number of recovered splenocytes was significantly reduced related to the dose level at all dose groups.

**Immune effects:**

Following four weeks of oral benzene treatment via the drinking water, the proliferative response of either mitogen-stimulated or nonstimulated splenic lymphocytes were elevated in male CD-1 mice at 8 mg/kg bw and depressed at 40 and 180 mg/kg bw/d (Hsieh et al. 1988b). This biphasic alterations in proliferation of T- and B-lymphocytes was observed using LPS, PWM, ConA and PHA.

Hsieh et al. (1988b) assessed the primary antibody response to SRBC after benzene exposure. The number of PFCs in male CD-1 mice receiving 40 and 180 mg/kg bw/d of benzene was reduced, when expressed on either specific activity of PFC/106 spleen cells or whole spleen basis. However, there were more PFC per 106 spleen cells at the low level of benzene. The titers of SRBC antibodies corresponded to the numbers of PFC.

Following four weeks of oral benzene treatment via the drinking water, the proliferative response of either mitogen-stimulated or nonstimulated splenic lymphocytes were elevated in male CD-1 mice at 8 mg/kg bw and depressed at 40 and 180 mg/kg bw/d (Hsieh et al. 1988b). This biphasic alterations in proliferation of T- and B-lymphocytes was observed using LPS, PWM, ConA and PHA.

In in vitro studies supernatants from splenic T-lymphocyte cultures stimulated with ConA were assayed for Interleukin-2 (IL-2) content by their ability to enhance proliferation of the murine T-helper cell line HT-2. Splenic IL-2 production was suppressed in the 40 and 180 mg/kg bw treated benzene groups (Hsieh et al. 1991).

In a further study, cell mediated immunity was measured in splenic lymphocytes of 4 weeks orally exposed male CD-1 mice by mixed-lymphocyte culture response to allogenic cells and cytotoxic T-lymphocyte (CTL) activity to YAC-1 tumor cells. Both immune reactions were inhibited at benzene doses of 40 and 180 mg/kg bw/d, but increased in the 8 mg/kg bw/d dose group (Hsieh et al. 1988b).

Hsieh et al. (1988a) observed increased concentrations of norepinephrine (NE) in the hypothalamus, medulla oblongata and cerebellum of CD-1 mice fed continuously with drinking water containing 31, 166 and 790 mg/l benzene for four weeks. Dopamin (DA) concentrations increased significantly in the hypothalamus and corpus striatum. Increases of several catecholamine metabolites and the indoleamine serotonin (5-HT) were seen in a number of brain regions. The authors concluded that benzene induced increased rates of synthesis and catabolism of neurotransmitters NE, DA, and 5-HT. Besides of direct toxic effects on the immune system, the increases in brain catecholamines can act indirectly on the immune system via hypothalamus-pituitary-adrenal axis. Increased metabolisms of catecholamines can result in increased adrenal corticosteroid levels.

Oral administration to mice at doses from 8 mg/kg bw/d, for four weeks; induced increased catecholamine concentrations of the brain, increased adrenocorticotropin (ACTH) and corticosterone release into the blood (Hsieh et al. 1991). An indirect action on the immune system via the hypothalamus-pituitary-adrenal axis was supposed. The oral studies did not include any data on behavioural dysfunctions or morphological abnormalities.

**Source:** BP Chemicals Ltd LONDON;  
German Rapporteur

**Test substance:** Laboratory reagent; 99.9% pure.  
**Flag:** Risk Assessment  
06-JUL-2005 (536) (537) (538)

**Species:** mouse **Sex:** male/female  
**Strain:** B6C3F1  
**Route of administration:** gavage  
**Exposure period:** 17 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** none  
**Doses:** 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 mice/sex/dose  
**Control Group:** yes, concurrent vehicle  
**NOAEL:** 25 mg/kg bw  
**LOAEL:** mg/kg bw

**Method:** other  
**GLP:** yes

**Remark:** Groups of ten or 15 animals of each sex were used.

Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim-kill and the top-dose groups were removed and examined histopathologically; spleens were examined in all dose groups.

**Result:** No compound-related deaths. Mean body weight gain was dose-dependently decreased in both the males and females at 100 mg/kg bw or more. Tremors were observed intermittently in the 400 and 600 mg/kg bw groups, those during the last 3 weeks of the study being more pronounced in the males than females. At day 60, no changes in the white blood cell or lymphocyte counts were seen in either the male or female animals given 200 or 600 mg/kg bw. Both counts were dose-dependently and significantly ( $p < 0.05$ ) decreased at the end of the study in the males at 50 mg/kg bw or more and in the females at 600 mg/kg bw in the case of the white blood cells and at 400 mg/kg bw or more in the case of the lymphocytes. No compound-related histopathological changes were observed.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.7% pure.  
**Flag:** Risk Assessment  
07-JUL-2005 (540) (843)

**Species:** mouse **Sex:** male/female  
**Strain:** B6C3F1  
**Route of administration:** gavage  
**Exposure period:** 103 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 1 week  
**Doses:** 25, 50, 100 mg/kg bw/day; 60 mice/sex/dose  
**Control Group:** yes, concurrent vehicle  
**LOAEL:** 25 mg/kg bw

**Method:** other  
**GLP:** yes  
**Test substance:** other TS

**Method:** In this cancer study, mice of each sex were administered to 0, 25, 50, or 100 mg/kg bw benzene by gavage, 5 d/w for 103 weeks. Blood was withdrawn from 10 animals/sex/group at 12, 15, 18, and 21 months. Additional groups of 10 animals of each sex and species were treated at the same doses for 51 weeks, blood was withdrawn at 0, 3, 6, 9, and 12 months.

**Remark:** Benzene administered in corn-oil. Blood was drawn from ten randomly preselected animals from each sex and dose group at 0, 3, 6, 9, 12, 15, 18 and 21 months and from all animals at the terminal kill at 24 months. Ten animals of each sex and group were killed after 51 weeks and necropsies were performed. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.

**Result:** A decrease in weight gain was noted in the high-dosed males and females after 47 and 87 weeks respectively. No other clinical signs related to benzene exposure were seen. The survival rates were significantly decreased in the high-dosed male and female animals. Various non-neoplastic lesions were found in the zymbal gland, preputial gland, harderian gland, adrenal gland, ovary, lung, spleen, bone marrow and forestomach of the treated animals.

Hematologic effects were limited to lymphocytopenia and associated leukocytopenia in all mouse dose groups (males from 3 to 18 months, female mice from 12 to 18 months). Benzene increased the frequency of micronucleated normochromatic peripheral erythrocytes in male and female mice of all dose groups, males were more sensitive than females.

Hematopoietic hyperplasia in the bone marrow and splenic hematopoiesis was observed in all dosed mice groups. (Tumor data and survival rates were reviewed in section 5.7 for carcinogenic effects. LOEL related to noncarcinogenic effects.

**Source:** BP Chemicals Ltd LONDON; German Rapporteur  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.7% pure.  
**Flag:** Risk Assessment

07-JUL-2005

(540) (844)

**Species:** rat **Sex:** male/female  
**Strain:** Fischer 344  
**Route of administration:** gavage  
**Exposure period:** 17 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** none  
**Doses:** 25, 50, 100, 200, 400, 600 mg/kg bw/day; 10 or 15 rats/sex/dose  
**Control Group:** yes, concurrent vehicle  
**LOAEL:** = 25 mg/kg bw

**Method:** other  
**GLP:** yes  
**Test substance:** other TS

**Method:** In a National Toxicology Program (NTP) study (NTP 1986; Huff et al. 1989), Fischer 344 rats were evaluated to cumulative toxicity of benzene in 17 week studies and two-year studies. In the seventeen-week study, groups of 10 rats were administered 0, 25, 50, 100 or 400 mg/kg benzene in corn oil by gavage. Groups of 15 rats were administered 0, 200, or 600 mg/kg bw/d, 5 animals of each of these groups were killed on day 60.

**Remark:** Benzene administered in corn oil. Animals were checked twice daily and weights recorded weekly. Five animals of each sex from the control, 200 and 600 mg/kg bw groups were killed on day 60. Survivors from all groups killed at end of the study period and haematologic analyses were performed on five animals from each group. At necropsy, a large number of organs from animals from the control, the interim-kill and the top-dose groups were removed and examined histopathologically; spleens were examined in all dose groups.  
in males: NOAEL 100 mg/kg bw/d, in females: LOAEL: 25 mg/kg bw/d

**Result:** No compound-related deaths. Mean body weight gain was dose-dependently decreased in both the males and females at 200 mg/kg bw or more. White blood cell and, in particular, lymphocyte counts were decreased significantly ( $p < 0.05$ ) on day 60 in both the male and female groups given 200 and 600 mg/kg bw and on day 120 in all the female treated groups and the top two dosed male groups. A significant increase ( $p < 0.05$ ) in white blood cell count was recorded on day 120 in the 25 mg/kg bw male group.

In the spleen, lymphoid depletion of B-cells was evident in both sexes at 200 mg/kg and above, increased extramedullary hematopoiesis was seen in male and female rats at 600 mg/kg bw/d.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.7% pure.  
**Flag:** Risk Assessment

07-JUL-2005

(540) (843)

**Species:** rat **Sex:** male/female  
**Strain:** Fischer 344  
**Route of administration:** gavage  
**Exposure period:** 103 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 1 week  
**Doses:** 50, 100, 200 mg/kg bw/day for males, 25, 50, 100 mg/kg bw/day for females; 60 rats/sex/dose  
**Control Group:** yes, concurrent vehicle  
**LOAEL:** 25 mg/kg bw

**Method:** other  
**GLP:** yes

**Method:** In the cancer study 50 male rats were administered to 0, 50, 100, or 200 mg/kg bw and female rats were administered to 0, 25, 50, or 100 mg/kg bw benzene in corn-oil by gavage, 5 d/w for 103 weeks. Blood was withdrawn from 10 animals/sex/group at 12, 15, 18, and 21 months. Additional groups of 10 animals of each sex and species were treated at the same doses for 51 weeks and necropsies were performed; blood was withdrawn at 0, 3, 6, 9, and 12 months. Animals were observed twice daily and weighed weekly for 13 wk and monthly thereafter.

**Remark:** LOAEL 25 mg/kg bw/d for females, 50 mg/kg bw/d for males  
**Result:** Weight gain reductions occurred in mid and high dose males rats, and high dose female rats. Hematologic effects were limited to lymphocytopenia and associated leukocytopenia in all male rat groups from 3 to 18 months; a similar but less pronounced response was observed in dosed female rats during the same time period. The frequency of micronucleated normochromatic peripheral erythrocytes was not examined in rats. Histopathology revealed increased incidences at all dose groups of lymphoid depletion in the spleen (male and female rats) and the thymus (male rats). (Tumor data and survival rates were reviewed in section 5.7 carcinogenicity)

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** >99.7% pure.  
**Flag:** Risk Assessment

07-JUL-2005

(540) (843)

**Species:** rabbit **Sex:** female  
**Strain:** New Zealand white  
**Route of administration:** s.c.  
**Exposure period:** 10 days  
**Frequency of treatment:** daily  
**Post exposure period:** 30 days  
**Doses:** 0.25, 0.5 ml/kg bw/day (converts to 219.5, 439 mg/kg bw/day); 8 or 9 rabbits/dose  
**Control Group:** yes, concurrent vehicle  
**NOAEL:** mg/kg bw  
**LOAEL:** 219 mg/kg bw

**Method:** other  
**GLP:** no data

**Method:** A group of 9 rabbits (no data on sex) was administered subcutaneously to 0.5 ml/kg bw/d of benzene on 10 consecutive days, another group of 8 animals received 0.25 ml/kg bw/d (Irons and Moore 1980). 4, respectively 2 animals were allowed to recover for up to 30 days.

**Result:** Benzene administered as a 50% (v/v) solution in corn oil. Blood was collected from the middle ear artery. Treatment resulted in a dose-related rapid loss of circulating lymphocytes up to 80%. At the end of the treatment period, circulating immunoglobulin-positive lymphocytes representing B-lymphocytes were depressed. Recovery of lowered values was incomplete at the end of the recovery period. The percentage of immunoglobulin-negative lymphocytes at the high dose level were also decreased in relation to the baseline values on day 0. The authors concluded that circulating lymphocytes were decreased due to a selective toxic effect on B-cells.

**Source:** BP Chemicals Ltd LONDON; German Rapporteur  
**Test substance:** Laboratory reagent grade benzene tested.  
**Flag:** Risk Assessment

27-OCT-2000

(568)

**Species:** mouse **Sex:** male  
**Strain:** other: C57B1/6J  
**Route of administration:** i.p.  
**Exposure period:** 2 d  
**Frequency of treatment:** twice a day  
**Post exposure period:** up to 7 d  
**Doses:** 600 mg/kg bw  
**Control Group:** yes  
**LOAEL:** 600 mg/kg bw

**Method:** other

**Result:** Short-term treatment with 600 mg/kg bw of benzene, twice a day on 2 days, injected intraperitoneally to four male C57B1/6J mice caused a significant depression of the total number of nucleated bone marrow cells per femur on day 3. Depression of the nucleated erythroid cells started at day 3 and remained constant until day 7 of monitoring. Reduction of lymphocyte counts also started on day 3 and progressively decreased until day 7. Conversely, the numbers of intermediate and terminally differentiated granulocytes progressively increased over 7 days.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(824)

**Species:** mouse **Sex:** male  
**Strain:** other: C57B1/6J  
**Route of administration:** inhalation  
**Exposure period:** 20 weeks  
**Frequency of treatment:** 6 h/d, 5 d/w  
**Doses:** 10, 30, 100 ppm  
**Control Group:** yes  
**NOAEL:** 100 ppm

**Method:** other

**Method:** Characterisation of spleen cell populations in five animals/group

**Remark:** NOAEL for selected parameter

**Result:** The data show that the relative proportions of splenic leukocytes, the percentages of splenic T-cell subsets and the ratio of splenic helper/suppressor cells are not effected.

Inhalative exposure of male C57B1/6J mice to 100 ppm benzene (320 mg/m<sup>3</sup>) (6 hr/d for 5d/w) on 10 consecutive days were performed before starting the tumor cell inoculation. Reduced tumor lytic abilities of splenic cytotoxic T-lymphocytes were demonstrated. Splenic T-lymphocytes taken from mice treated with 10 ppm (960 mg/m<sup>3</sup>) and 100 ppm for 20 days showed a delayed mixed lymphocyte reaction (MLR) to alloantigens. This delayed MLR response was not due to the presence of benzene-induced suppressor cells. The authors suggested that benzene impaired the functional abilities of alloreactive T-cells.

Reduced tumor resistance mediated via T-lymphocytes was observed in 9 out of 10 male C57Bl/6J mice exposed to 100 ppm benzene (320 mg/m<sup>3</sup>) for a total of 100 days (6 hr/d, 5 d/w, 20 weeks) and challenged with 10.00 polyoma virus-induced tumor cells/mouse. These mice developed tumors that were lethal. Lethal tumor incidences in air controls and mice exposed to 10 or 30 ppm benzene concentrations were 3/10 or less.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(960)

**Species:** mouse **Sex:** male  
**Strain:** other: C57Bl/6  
**Route of administration:** inhalation  
**Exposure period:** 5 d prior to infection, additional subgroup with postinfectious exposure on 7 d  
**Frequency of treatment:** 6 h/d,  
**Post exposure period:** 7 d  
**Doses:** 10, 30, 100, 300 ppm followed by infection with *Listeria monocytogenes*  
**NOAEL:** 10 ppm

**Method:** other

**Method:** Host resistance test, 5-7 animals/group

**Result:** Continuous exposure to concentrations as low as 30 ppm (96 mg/m<sup>3</sup>) of benzene resulted in a delay in immune response of T-cells and macrophages after induction of bacterial infection in C57BL/6 mice (Rosenthal and Snyder 1985). Preexposure of male C57Bl/6J mice (5-7 animals/group) to benzene at 10, 30, 100, or 300 ppm for 5 days followed by infection with *Listeria monocytogenes* with continuous exposure on 7 days or without continuing the exposure increased the bacterial counts in mice of the 300 ppm group of the preexposure group and in mice at 30, 100, and 300 ppm of the continuous exposure groups on day 4, but not days 1 or 7. Nucleated cells, lymphocytes, T- and B-lymphocytes and monocytic/macrophagic cells per spleen increased from day 1 to day 7 of infection in air control groups. Significant depressions of the mentioned cell types except the monocytic/macrophagic cells were observed in each exposure regimens at 30 ppm or higher concentrations from day 1 through day 7 of bacterial infection.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(959)

**Species:** mouse **Sex:** female  
**Strain:** other: BNL  
**Route of administration:** inhalation  
**Exposure period:** 5, 10 or 20 exposures at 50 ppm and 200 ppm, 5, 12, or 22 exposures at 400 ppm  
**Frequency of treatment:** 6 h/d, 5 d/wk  
**Doses:** 50, 200, 400 ppm (160, 640, 1280 mg/m<sup>3</sup>)

**Method:** other

**Method:** Immune response to toxin  
**Result:** Concentration of 200 ppm (640 mg/m<sup>3</sup>) for 10 or 20 exposures or 400 ppm (1280 mg/m<sup>3</sup>) for 5, 12, or 22 exposures (6 hr/d, 5 d/w) suppressed the T-cell dependent primary antibody response to tetanus toxin in female BNL mice (15 animals/group) on day 21 after immunisation (Stoner et al. 1981). No effect at 50 ppm (160 mg/m<sup>3</sup>) after 5, 10, or 20 exposures and at 200 ppm after 5 exposures was measured.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(1093)

**Species:** mouse **Sex:**  
**Strain:** Balb/c  
**Route of administration:** other: subcutaneous  
**Exposure period:** 3 days  
**Doses:** 880 mg/kg/d

**Method:** other

**Test substance:** other TS: benzene, not specified

**Result:** Subcutaneous injection on 3 days with 880 mg/kg bw/d of benzene or a combination of 50 mg/kg bw phenol and hydroquinone to BALB/c mice (no data on number and sex of experimental animals) were found to activate bone marrow derived macrophages and granulocytes measured as an increased production of hydrogen peroxide after stimulation. The number of cells recovered from the bone marrow of the femur and tibia was decreased in each of the treatment groups to 30-40% of the control values.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(668)

**Species:** rat **Sex:** male/female  
**Strain:** Wistar  
**Route of administration:** inhalation  
**Exposure period:** up to 212 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Post exposure period:** 18-22 hours  
**Doses:** 0.28, 7, 14, 21, 30 mg/l; 10-25 rats/sex/dose  
**Control Group:** yes, concurrent no treatment  
**NOAEL:** < .28 mg/l  
**LOAEL:** = .28 mg/l

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten to 25 male and female animals were used.

All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

TS concentration was analysed (no data about method) and adjusted, variation of concentration 10%. Males and females tested only at the low dose, other groups only males.

**Result:** Increased spleen weight and unspecified changes in blood histopathology including leucopenia were recorded at the lowest dose level with, in addition, narcosis, growth depression and unspecified spleen and bone marrow histopathology at 7 mg/l (exposure period 221 d) or more (up to 93 d). Mortality rate increased at  $\geq$  14 mg/l and increased testis weight and unspecified liver histopathology occurred at 21 mg/l (exposure period 93 d).

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure.

06-JUL-2005

(1263)

**Species:** rat **Sex:** female  
**Strain:** Wistar  
**Route of administration:** gavage  
**Exposure period:** 6 months  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 18-22 hours  
**Doses:** 1, 10, 50, 100 mg/kg bw/day; 10 rats/dose  
**Control Group:** yes, concurrent vehicle  
**NOAEL:** = 1 mg/kg bw  
**LOAEL:** = 10 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene given as an olive-oil solution. Haematological examinations made on selected animals of each group at varying intervals. Animals were weighed at regular intervals and frequent observations were made of their general appearance and behaviour. Records were kept of food consumption and mortality. Gross appearance of lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Blood was obtained from selected animals of each group at time of autopsy. Histopathological examination of above organs and adrenals, pancreas and femoral bone marrow made.

**Result:** Very slight leucopenia was seen at 10 mg/kg bw/day, with leucopenia and erythrocytopenia occurring at the top two doses. No further results were reported.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure.

06-JUL-2005

(1264)

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** for life  
**Frequency of treatment:** 6 hr/day, 5 days/week  
**Doses:** 300 ppm  
**Control Group:** yes, concurrent no treatment

**Method:** other: see ref.

**Year:** 1982

**GLP:** no data

**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID

26-OCT-2000

(548)

**Species:** rabbit **Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure period:** 243 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Post exposure period:** 18-22 hours  
**Doses:** 0.26 mg/l; 1 or 2 rabbits tested  
**Control Group:** yes, concurrent no treatment  
**NOAEL:** < .26 mg/l  
**LOAEL:** = .26 mg/l

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

**Result:** Unspecified histopathological changes in the kidney, testes and blood (including leucopenia) were reported.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** 99.98% pure.

13-DEC-1996

(1264)

**Species:** rabbit **Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure period:** 243 days  
**Frequency of treatment:** 7 hours per day, 5 days per week  
**Post exposure period:** 18-22 hours  
**Doses:** 0.26 mg/l; 1 or 2 rabbits tested  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 3 (not valid)  
Significant methodological deficiencies  
one dose, low number of animals, one sex, no clinical biochemistry, limited hematology, limited pathology, no further data about results of enforced investigations  
The animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy. TS concentration analysed (no

further data) and adjusted, 10% variation.

**Result:** Unspecified histopathological changes in the kidney, testes and blood (including leucopenia) were reported.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure. (1264)

06-JAN-1997

**Species:** rabbit **Sex:** female

**Strain:** New Zealand white

**Route of administration:** s.c.

**Exposure period:** 10 days

**Frequency of treatment:** once daily

**Post exposure period:** up to 40 days

**Doses:** 0, 0.25, 0.5 ml/kg bw/day (converts to 0, 219.5, 439 mg/kg bw/day); 6, 8 and 9 rabbits/dose, respectively

**Control Group:** yes, concurrent vehicle

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Benzene administered as a 50% (v/v) solution in corn oil. Blood was collected from the middle ear artery at different intervals (day 0 and 1-40). Reliability: 3 (not valid) Unsuitable test system only effects on lymphocytes studied, no other toxicological effects reported

**Result:** A dose- and time-dependent decrease in circulating lymphocytes (80% reduction in the high dose group, 35% at 0.25 ml/kg) which partially recovered 30 days after treatment, was noted. Circulating monocytes were significantly decreased in the top-dosed animals at day 10. Immunoglobulin surface receptor-negative cells (presumably T-lymphocytes) were relatively unaffected.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade benzene, thiophene-free, checked by GCMS (568)

06-JAN-1997

**Species:** guinea pig **Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure period:** up to 269 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Post exposure period:** 18-22 hours  
**Doses:** 0.28 mg/l; 5 or 10 guinea pigs tested  
**Control Group:** yes, concurrent no treatment  
**NOAEL:** < .28 mg/l  
**LOAEL:** = .28 mg/l

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

**Result:** A group of five or ten animals used. Increased kidney, spleen and testes weights, growth depression and unspecified histopathological changes in the bone marrow and blood (including leucopenia) were noted.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** 99.98% pure.

13-DEC-1996

(1264)

**Species:** guinea pig **Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure period:** 32 or 269 days  
**Frequency of treatment:** 7 hours per day, 5 days per week  
**Post exposure period:** 18-22 hours  
**Doses:** 0.28 mg/l; 5 to 10 guinea pigs tested  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 3 (not valid)  
Significant methodological deficiencies  
one dose, low number of animals, one sex, no clinical biochemistry, limited hematology, limited pathology, no further data about results of enforced investigations, no statistical evaluation  
TS concentration analysed (no further data) and adjusted, variation was 10% of desired concentration.  
All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. The gross appearance of the

lungs, heart, liver, kidneys, spleen and testes was observed and these organs weighed. Histopathological examination of these organs and the adrenals, pancreas and femoral bone marrow was made. Blood was collected from selected animals of each group at autopsy.

**Result:** After 269 d increased spleen and testes weights, growth depression and unspecified histopathological changes in the bone marrow and blood (including leucopenia) were noted. After 32 d increased kidney weight and unspecified histopathological changes in blood (including leucopenia).

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure.

06-JAN-1997 (1264)

### 5.5 Genetic Toxicity 'in Vitro'

**Type:** Ames test

**System of testing:** Salmonella typhimurium strains TA97, 98, 100, 1535

**Concentration:** 0, 0.01, 0.033, 0.1, 0.333, 1.0 mg/plate

**Metabolic activation:** with and without

**Result:** negative

**Method:** OECD Guide-line 471

**Year:** 1983

**GLP:** no data

**Test substance:** other TS

**Remark:** Metabolic activation system was Aroclor-1254-induced S9 from male Sprague-Dawley rats and Syrian hamsters. Benzene tested at a minimum of five doses up to a toxic dose or the limit of solubility, to a maximum dose of 10 mg/plate. Triplicate plates were used. The concentration of S9 in the S9 mix was 10 or 30%. The positive controls were 9-aminoacridine, 2-aminoanthracene, 4-nitro-O-phenylenediamine and sodium azide. Several other investigators have also reported negative findings in S. typhimurium reverse mutation assays using several tester strains. Some of these include Cotruvo J.A. et al. Ann. N.Y. Acad. Sci. 298, 124-140, 1977; Florin I. et al. Toxicology 15, 219-232, 1980; Matsushima T. et al. Prog. Mutation Res. 5, 181-186, 1985; Shimizu M. et al. Mutation Res. 116, 217-238, 1983.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** 99% pure.

13-DEC-1996 (1305)

**Type:** Salmonella typhimurium reverse mutation assay  
**System of testing:** Salmonella typhimurium strain TM667  
**Concentration:** <=13mM (1014 mg/l)  
**Metabolic activation:** with  
**Result:** negative

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The mutation assay was carried out according to Skopek T.R. et al. Proc. natn. Acad. Sci. U.S.A. 75, 410-414 & 4470-4473, 1978. Bacteria exposed for 2 hr in the presence of 10% of a postmitochondrial supernatant, prepared as a 25% liver homogenate of phenobarbital- or Aroclor-pretreated male Sprague-Dawley rats. Resistance to 8-azaguanine was used as the genetic marker.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Supplied by Aldrich Chemical Co., Milwaukee, Wisconsin; purity unspecified.

13-DEC-1996

(591)

**Type:** Salmonella typhimurium reverse mutation assay  
**System of testing:** Salmonella typhimurium strains TA97, 98, 100, 102, 104, 1535  
**Concentration:** 3-1000ppm (converts to 0.009- 3.26 mg/l)  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was found to be mutagenic in the presence but not the absence of S9. Strain TA1535 was the most responsive. A benzene concentration as low as 0.03 mg/l caused a two-fold increase in the number of mutants above the control. In this same strain, the metabolites trans-benzene-1,2-dihydrodiol in the presence of S9 and anti-benzene-diol-epoxide and syn-benzene-diol-epoxide in the absence of S9 induced mutations. No other metabolite, including catechol to which trans-benzene-1,2-dihydrodiol is converted by cytosolic dihydrodiol dehydrogenase, gave a positive result in strain TA1535. Mutagenic responses, some of them weak, were noted in other strains treated with 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, catechol, quinone, hydroquinone, syn-benzene-diol-epoxide and anti-benzene-diol-epoxide. Tests were conducted on benzene and its following metabolites: benzene oxide, phenol, hydroquinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxy-biphenyl, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-tri-hydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. S. typhimurium was exposed to benzene vapour in desiccators to allow for

longer exposure periods. S9 mix derived from rat and mouse liver homogenate was used as the activation system.

**Source:** BP Chemicals Ltd LONDON (416)  
13-DEC-1996

**Type:** Mammalian cell gene mutation assay  
**System of testing:** Chinese hamster V79 cells  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Mutations to 6-thioguanine resistance were induced by quinone (a potent inducer), anti-benzene-diol-epoxide, hydroquinone, catechol, trans-benzene-1,2-dihydrodiol and 1,2,4-trihydroxybenzene. The cytotoxicity and potency of the mutagenic effect varied between the chemicals. Phenol, the dihydroxybiphenyls and duroquinone showed no mutagenic effect. Anti-benzene-diol epoxide induced a significant but weak mutation to ouabain resistance.

Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxybiphenyl, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone and anti-benzene-diol-epoxide were also included. Compounds were tested up to their cytotoxic limits. Cells were mixed with the test compound for 24 hours and mutants were determined after an expression period of 6 days.

**Source:** BP Chemicals Ltd LONDON (416)  
13-DEC-1996

**Type:** HGPRT assay  
**System of testing:** Chinese hamster V79 cells  
**Concentration:** 0.1, 0.3, 1.0, 2.0 mg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Preliminary cytotoxicity studies were carried out using this same cell-line, with and without 5% foetal calf serum. Benzene was added over a concentration range of 5 µg/ml to 10 mg/ml in the presence and absence of a metabolic activation system (S9 mix). Cells were examined microscopically at intervals up to 4 hr for evidence of damage. Based on the results of this study, a dose range was chosen for use in assays for inhibition of colony-forming ability. This was conducted in the presence and absence of serum and the presence and absence of S9 mix for periods of up to 4 hr. For the mutagenicity assay, the cells were exposed to benzene for 1 hr in the absence of

serum and the presence and absence of S9 mix. Cyclophosphamide and ethylmethanesulphonate were used as positive controls, in the presence and absence of S9 mix respectively. The concentrations of benzene tested included at least one dose that induced measurable cytotoxicity and one higher dose. The S9 mix used was derived from livers of male albino rats treated with phenobarbital and  $\beta$ -naphthoflavone.

The maximum non-toxic benzene dose in the cytotoxicity assay was 0.31 mg/ml when tested for 4 hr in the presence of serum and the presence and absence of S9 or in the absence of serum and the presence of S9. When tested without S9, in the absence of serum, the maximum non-toxic dose was 0.63 mg/ml. In the colony forming assay, the highest non-toxic dose when tested with and without serum and with and without S9 mix was 0.003 mg/ml and the concentration which killed 50% of the cells in the presence of serum and the absence and presence of S9 mix was 1 mg/ml and >1 mg/ml in the absence of serum. In the mutagenicity assay, the mutation frequency was evaluated 5 and 8 days after exposure. No mutagenic activity was found. A similar study conducted at benzene concentrations of 0.1 to 0.5 mg/ml and in the absence of a metabolic activation system, also failed to show any mutagenic activity (Elmore E. et al. Prog. Mutation Res. 5, 597-612, 1985).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(368)

**Type:** HGPRT assay  
**System of testing:** Chinese hamster V79 cells  
**Concentration:** 1, 10, 50, 100, 500, 1000  $\mu$ g/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** A preliminary cytotoxicity study was carried out using this same cell line treated with 1, 10, 50, 100 and 500  $\mu$ g/ml for 3 hours. The treated cells were then rinsed with Hanks' solution and incubated in normal medium for a further 7 days before the colony-forming activity was evaluated. The mutagenic activity was determined by the 'replating' method. Cells were treated for 3 hours with benzene, rinsed with Hanks' solution and then incubated in normal medium for 6 days. They were then treated with trypsin, replated in medium containing 6-thioguanine and incubated for 14 days. The number of 6-thioguanine-resistant colonies was scored. The assay was conducted in the absence and presence of a metabolic activation system (S9 mix) which had been obtained from male Sprague-Dawley rats treated with an intraperitoneal injection of phenobarbital and 5,6-benzoflavone. Benzene was dissolved in dimethylsulphoxide before use and diluted with Hanks'

solution to obtain the appropriate concentration for treatment of cells. The vehicle alone was tested as the 0 µg benzene/ml level and ethyl-methanesulphonate was the positive control chemical.

Cells treated with benzene at 100 and 500 µg/ml had slightly decreased colony-forming activities, showing slight cytotoxicity. A slight mutagenic effect was noted at 50 and 100 µg/ml, tested in the absence of S9 mix. No mutations were noted at any concentration in the presence of S9 mix.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(648)

**Type:** Mouse lymphoma assay  
**System of testing:** L5178Y mouse lymphoma cells  
**Concentration:** 66, 88, 132, 176, 352, 528 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Cells treated for 2 hours in the absence and presence of S9 showed 66.6 and 1% survival respectively at a benzene concentration of 352 µg/ml. The cells were completely killed at concentrations of 704 and 528 µg/ml when treated in the absence and presence of S9 respectively. In the mutagenicity assays, benzene was tested at concentrations of 88, 176, 352 and 528 µg/ml in the absence of any activation; 88, 176 and 352 µg/ml in the presence of S9; and at 66 and 132 µg/ml in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. No evidence of mutagenicity at the thymidine kinase locus or the hypoxanthine guanine phosphoribosyltransferase locus was seen in any of the systems. Similar negative findings have been reported by Amacher D.E. & Turner G.N. Prog. Mutation Res. 5, 487-496, 1985; Myhr B et al. *ibid.* 5, 555-568, 1985; Oberly T.J. et al. Mutation Res. 125, 291-306, 1984. Preliminary cytotoxicity tests were conducted in the presence and absence of metabolic activation. The metabolic activation systems were liver enzymes from Aroclor-1254-induced male SPF Wistar rats (S9 mix), primary chick embryo hepatocytes, and Syrian hamster embryo cells. The results of this test were used to select the concentrations to be used in the mutagenicity assay, with the lowest concentration showing no toxicity and the highest giving a survival of about 15% immediately after treatment. In the mutagenicity test, cells were treated for 2 hours in the absence or presence of S9 and for 20 hours in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. After treatment, the cells were seeded for survival or propagated for several days in standard medium to allow for the expression of induced mutants. Cloning efficiency and selection of mutants were made and mutant frequency assessed. Benzene was dissolved

or diluted to the appropriate test concentration in dimethylsulphoxide and this solvent was included in each experiment at 1% as the negative control. Ethylnitrosourea, diethylnitrosamine and benzo(a)pyrene acted as the positive controls in the experiments with no activation, activated with S9 and activated with Syrian hamster-embryo cells and primary chick-embryo hepatocytes respectively.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(624)

**Type:** Mouse lymphoma assay  
**System of testing:** L5178Y mouse lymphoma cells  
**Concentration:** 500-1200 µg/ml and 100-800 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Based on former toxicity data, concentrations of 500-1200 µg/ml and 100-800 µg/ml were selected for the nonactivated and activated tests respectively. In the absence of S9, 1000 µg/ml killed all the test culture and 49% survival was noted at 600 µg/ml. In the presence of S9, 8% survival was seen at a benzene concentration of 800 µg/ml and 45% survival occurred at 100 µg/ml. In the mutagenicity test, benzene was found not to be mutagenic in the absence of S9. In the presence of S9, cultures exposed to 100, 300, 400 and 500 µg/ml showed 2-3-fold increases in mutant frequency over the background. Survival in these cultures exceeded 10% and benzene was, therefore, judged to be mutagenic in the presence of activation. A similar result was reported by Styles J.A. et al. Prog. Mutation Res. 5, 587-596, 1985. In the presence of S9, benzene produced an unequivocal positive result at the oua locus and a weak result at the TK locus. No such effect was found in the absence of S9 but this was thought to be due to excessive toxicity of benzene. Benzene was tested dissolved in dimethylsulphoxide. The metabolic activation system (S9) was prepared from the livers of male Fischer 344 rats pretreated with Aroclor-1254. Preliminary toxicity studies previously conducted at the same laboratory were used to determine the concentrations to be used in the mutagenicity assay. The highest dose selected was that concentration causing a 50-90% inhibition of growth in the preliminary study. In the mutagenicity study, cells were treated with benzene for 4 hours, washed, resuspended in normal medium and incubated for a further 48 hours. Mutant frequency was then evaluated after a further 12-day incubation period. This procedure was repeated with the addition of S9. Dimethylsulphoxide (1%) was used as the negative solvent control and ethylmethanesulphonate and 3-methyl-cholanthrene as the positive controls in the nonactivated and activated tests respectively.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996 (850)

**Type:** Mouse lymphoma assay  
**System of testing:** L5178Y mouse lymphoma cells  
**Concentration:** 12.5-200 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**Year:** 1976  
**GLP:** no data  
**Test substance:** other TS

**Remark:** From the preliminary study, a dose range of 12.5-200 µg/ml was determined. The mutant frequency was statistically significantly increased in the 6-thioguanine test in the absence of S9. The method of Cole J. et al. Mutation Res. 41, 377-386, 1976 was followed. Benzene was dissolved in dimethylsulphoxide and diluted to obtain the required concentration. Cells were treated in the presence and absence of S9 mix (derived from Aroclor-1254-induced rats) for 2 hours. Survival was determined initially at the various concentrations tested without S9. In the mutagenicity assay, after treatment, the cells were cultured for 48 hours, mixed with ouabain and incubated for 2 weeks or cultured for 7 days, mixed with 6-thioguanine and incubated for 2 weeks. Benzo(a)pyrene and 4-nitroquinoline-1-oxide were the positive control chemicals for the assays conducted with and without S9 respectively. Dimethylsulphoxide was the negative solvent control.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade.  
13-DEC-1996 (394)

**Type:** Cytogenetic assay  
**System of testing:** human lymphocytes  
**Concentration:** 9, 44, 88 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In the cytotoxicity study, a 50-80% reduction in mitosis was seen in cultures treated with 88 µg/ml when compared with the negative control. The mutagenicity test was conducted at benzene concentrations of 9, 44 and 88 µg/ml. A dose-related increase in chromosomal aberrations was noted in both the male and female donated lymphocytes treated in the absence and presence of S9. The incidence of all aberrations, excluding chromatid gaps, was statistically significantly increased over the negative controls, with all

treatment regimes; this being more pronounced in the males than females. A second set of investigators reported chromosomal aberrations in human lymphocytes treated in the absence of metabolic activation (Morimoto K. Jap. J. ind. Hlth 18, 23-34, 1976), however, negative results in the absence of activation have also been reported (Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978). The method of Richardson C.R. & Wildgoose J. Mutation Res. 100, 287-293, 1982 was followed. Benzene was dissolved in dimethylsulphoxide before use and dimethyl-sulphoxide was used to treat the negative control cultures. Metabolic activation system was derived from the liver of Aroclor-1254-induced rats. Blood was withdrawn from a donor of each sex who had a previously established low incidence of chromosomal damage. Preliminary cytotoxicity tests were performed in the absence and presence of S9. In the chromosome aberration test, the lymphocyte cultures were treated with benzene, 44 hours after initiation for 3 hours. When activation was used, benzene and the relevant controls were incubated for 30 min with S9 before being added to the blood cultures. The cultures exposed to benzene were agitated throughout the exposure period to maintain homogeneity of the chemical through the culture. Mitomycin C was used as the positive control substance in the absence of S9 and cyclophosphamide in the presence of S9. Lymphocytes were treated with colchicine before analysis.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(526)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 200, 600, 1800 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 473  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In cultures fixed after 3 hours, increases in the frequency of chromosomal aberrations were seen in the absence and presence of S9 at 1800 and 600 µg/ml respectively. No such effects were found after 12 hours and after 18 hours, increases were observed at 200 and 600 µg/ml when in the presence of S9. A second study conducted on a Chinese hamster lung fibroblast cell line also showed benzene-induced chromosome aberrations when tested in the presence but not the absence of S9 (Ishidate M. Jr & Sofuni T. Prog. Mutation Res. 5, 427-432, 1985). The metabolic activation system (S9 mix) was prepared from the livers of phenobarbital- and β-naphthoflavone-induced male Sprague-Dawley/CD rats. Benzene was dissolved in dimethylsulphoxide to achieve the required concentration. The negative control consisted of both untreated and solvent treated cultures. Cyclophosphamide and aflatoxin B1 were

used as the positive controls. Benzene was tested at concentrations of 200, 600 and 1800 µg/ml, the top dose giving approximately 50% inhibition of the mitotic index. Cells were treated for 3 hours, the medium aspirated and cultures washed. Some cultures were fixed while others were resuspended in fresh medium and incubated for 6 or 12 hours. Colchicine was added to these cultures and 3 hours later they were fixed.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996 (867)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene failed to induce chromosome aberrations when in either the absence or presence of S9. Similar negative findings have also been reported in this same cell line by Natarajan A.T. et al. Prog. Mutation Res. 5, 433-437, 1985; in a Chinese hamster fibroblast cell line in the absence of a metabolic activation system by Danford N. *ibid.* 5, 397-411, 1985; and in Wistar rat liver cells in the absence of metabolic activation by Priston R.A.J. & Dean B.J. *ibid.* 5, 387-395, 1985. The method of Galloway S.M. et al. *Envir. Mutagen.* 7, 1-51, 1985 was followed. Benzene was dissolved in dimethylsulphoxide to the required test concentration. The metabolic activation system (S9) was prepared from Aroclor-1254-induced male Sprague-Dawley rats. The dose levels used in the study were selected based on the toxicity of benzene observed in concurrently run sister-chromatid exchange studies. In the absence of S9 treatment, cells were exposed to benzene for 10 hours. Colcemid was added 2-3 hours prior to cell harvest. In the presence of S9, cells were treated with benzene and S9 for 2 hours. The cells were then washed, returned to complete medium and incubated for 11 hours, colcemid being added 2 hours before harvest.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; >=99% pure.  
13-DEC-1996 (457) (459)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 1.33 mg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** After a treatment time of 1 hour, 20.9 and 33.0% survival was seen in the cells treated without and with S9 respectively. Single-strand breaks were greatly increased after 30 min, 1 and 2 hours treatment in the absence of S9; the increase being time-dependent. No such effect was noted in the presence of S9.

The metabolic activation system was the S9 fraction from Aroclor-1254-induced rat liver. Benzene was dissolved in dimethylsulphoxide. The cells were incubated in the presence of benzene for 30 min, 1 or 2 hours. Metabolic activation was only added in the 1 hour study. Survival of the cells was also checked. The single-strand DNA breaks were detected by high-salt alkaline sucrose-gradient sedimentation. Untreated cells acted as the negative control and ethylmethanesulphonate and diethyl-nitrosamine treated cells as the positive controls.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(655)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 0.39-7.81 mg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide before addition to the cells. Metabolic activation system was derived from the liver of Aroclor-1254 induced Sprague-Dawley rats (S9 mix). Cells were treated for 1 hour with benzene, washed and subjected to alkaline sucrose sedimentation analysis in order to assess the DNA single-strand breaks. Prior to testing benzene, its cytotoxicity was determined by examining inhibition of cell growth. The cytotoxicity threshold, that is, the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 µg/ml when tested in the presence and absence of S9. In the mutagenicity study, benzene was tested at levels of 0.39-7.81 mg/ml. No single-strand breaks were found at levels up to 2.34 mg/ml tested with or without S9. Above this, single-strand breaks increased but this was thought to be due to the toxic effects of benzene. Other investigators have reported

similar negative results using the alkaline elution technique in Chinese hamster V79 cells in the presence and absence of metabolic activation (Swenberg J.A. et al. Biochem. biophys. Res. Commun. 72, 732-738, 1976) and rat hepatocytes in the absence of activation (Bradley M.O. Prog. Mutation Res. 5, 353-357, 1985; Sina J.F. et al. Mutation Res. 113, 357-391, 1983).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; purity unspecified.  
13-DEC-1996 (305)

**Type:** Cytogenetic assay  
**System of testing:** Balb/c-3T3 mouse embryo cells  
**Concentration:** 0.5, 1.67, 2.0, 3.33, 4.0, 5.0 mg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** Directive 87/302/EEC, part B, p. 73  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** A cytotoxicity study was initially carried out to determine the benzene doses to be tested in the cell transformation assay. Doses causing 10-100% survival of the cells were used. Cells were treated with benzene for 72 hours, washed and incubated for a further 24 days. Transformed foci were scored microscopically. Primary rat-liver cells were prepared from Fischer-344 rats and lethally X-irradiated. This made up the activation system and was added to the mouse embryo cells for 3 hours before treatment with benzene for 48 hours. The cultures were then treated with 12-O-tetra-decenoylphorbol-13-acetate biweekly for 3 weeks, starting 1-2 days after benzene treatment. Dimethylnitrosamine and 3-methylcholanthrene were used as the positive controls in the tests conducted with and without the addition of the rat liver cells respectively. Untreated cells were the negative controls. The tests were conducted in closed tissue culture flasks. In the cytotoxicity test, 59% survival was seen at doses of 3.0 and 3.33 mg/ml in the absence and presence of activation respectively. All cells were killed at 6.0 mg/ml in the absence of activation and 0.3% survival was found at 5.0 mg/ml with activation. In the transformation assay, doses of 0.5, 2.0 and 4.0 mg/ml and 1.67, 3.33 and 5.0 mg/ml were tested without and with activation respectively. In the absence of activation, no transformation activity was found. In the presence of activation, statistically significant effects were noted at all dose levels, the increases in the numbers of transformed cell foci being dose-related. A second set of investigators also reported a lack of transforming activity in this cell line when benzene was tested in the absence of activation. The test was not conducted, in this case, with added activation (Rundell J.O. et al. Envir. Sci. Res. 27, 309-324, 1983).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; purity unspecified.

13-DEC-1996

(748)

**Type:** Cytogenetic assay  
**System of testing:** C3H/10T½ mouse embryonic fibroblast cells  
**Concentration:** 50, 80, 100, 160, 200, 320 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** Directive 87/302/EEC, part B, p. 73  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Based on the preliminary toxicity tests, benzene was tested at concentrations of 50, 100 and 200 µg/ml in the absence and 80, 160 and 320 µg/ml in the presence of S9. No transformed foci were identified at any benzene concentration in the presence or absence of S9. A similar negative result in this cell line in the absence of activation was reported by Nesnow S. et al. Prog. Mutation Res. 5, 659-664, 1985. Benzene was dissolved and diluted in dimethylsulphoxide before use. The metabolic activation system (S9) was obtained from the liver of Aroclor-1254-treated male rats. A dose-ranging study was conducted to determine the doses to be used in a preliminary cytotoxicity test. The latter was conducted to establish the range of concentrations to be used in the cell transformation assay. In the transformation assay, the cells were exposed to benzene for 24 hours in the presence or absence of S9 mix. One of the treated cultures/dose was used to assess cell survival in the study. In the remaining cultures, the medium was changed twice weekly until the cells reached confluence and weekly thereafter. After an 8-week incubation period, the cultures were stained and assessed for cell transformation. Dimethylsulphoxide was used as the negative control chemical and cyclophosphamide as the positive control in the presence of S9. In the absence of the activation mix, 3-methylcholanthrene acted as the positive control.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(669)

**Type:** Cytogenetic assay  
**System of testing:** Syrian hamster embryo (SHE) cells  
**Concentration:** 20-500 µg/ml  
**Metabolic activation:** without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Lethally X-irradiated SHE cells were seeded and 24 hours later target cells were added. After 24 hours, benzene was added and the cells were incubated for 6 days. The medium was then removed, the cells rinsed and benzene added for a

further 24 hours. The cells were then washed and transformation activity assessed. Benzene was dissolved in dimethylsulphoxide. Benzo(a)pyrene was used as the positive control.

Positive results were obtained at concentrations of 20 µg/ml and above. However, at concentrations higher than 300 µg/ml, the transformation frequency decreased. Similar positive results in the absence of activation have been reported in this cell line by Barrett J.C. & Lamb P.W. Prog. Mutation Res. 5, 623-628, 1985. Amacher and Zelljadt reported a low transformation frequency in SHE cells when tested in the absence of metabolic activation (Amacher D.E. & Zelljadt I. Carcinogenesis 4, 291-295, 1983) and a negative result has been reported in the SHE cell-transformation enhancement assay using SA7 viruses, again in the absence of activation (Hatch G.G. & Anderson T.M. Prog. Mutation Res. 5, 629-638, 1985).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
08-AUG-2000

(997)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 7.81-7810 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide before addition to the culture medium. Aroclor-1254-induced Sprague-Dawley rat-liver S9 was used as the metabolic activation system. A preliminary cytotoxicity test was conducted in which cells were incubated with a range of benzene concentrations with and without S9 for 24 hours. From this test, the concentrations to be used in the micronucleus assay were selected. In the micronucleus assay, cells were treated for 1 hour, washed and incubated in normal medium for 24 hours. The micronucleus frequency was then assessed. Historic dimethylsulphoxide control data were used as the background readings.

In the preliminary study, the cytotoxicity threshold, that is the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 µg/ml when in both the absence and presence of S9. In the micronucleus assay, no evidence of mutagenicity was seen with and without S9 at concentrations of 7.81 mg/ml to 7.81 µg/ml.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(305)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster V79 cells  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Induction of micronuclei was reported in cells treated with anti-benzene-diol-epoxide, quinone, hydroquinone, catechol, phenol, 1,2,4-trihydroxybenzene, 1,2,3-trihydroxybenzene and duroquinone. The potency of this effect varied between the chemicals. No such effect was seen with syn-benzene-diol-epoxide and trans-benzene-1,2-dihydrodiol. Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compound for 24 hours. They were then harvested and scored for micronuclei. Compounds were tested up to their cytotoxic limits.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

(416)

**Type:** Sister chromatid exchange assay  
**System of testing:** human peripheral lymphocytes  
**Concentration:** 16, 78, 391 mg/l  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** An increased number of SCEs were found in cultures treated in the presence of 10% S9 mix. In the absence of S9 and at S9 concentrations of 1 or 90% no increase in the frequency of SCEs was noted. 10% S9 mix is thought to be the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. When the cells were exposed to benzene concentrations of  $2 \times 10^{-4}$ ,  $1 \times 10^{-3}$  and  $5 \times 10^{-3}M$  (approximately 16, 78 and 391 mg/l), a dose-related increase in SCEs was seen when the appropriate activation concentration was used. S9 mix at 10-30% converted benzene into active forms that were cytotoxic and delayed cell turnover times. Further examination of this suggested that the metabolites responsible for cell division delay may be different from those which induce SCE. The addition of GSH to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S9 mix. The addition of GSH also completely prevented the induction of SCEs by catechol and hydroquinone, two major phenolic metabolites of benzene and potent inducers of SCEs.

Blood was obtained from healthy adult men. Benzene was dissolved in serum-free culture medium. Cells were suspended in the serum-free culture medium containing benzene and the metabolic activation system (S9 mix derived from rat liver) and incubated for 2 hours. The flask was agitated to ensure even distribution of active metabolites among the cells. After incubation, the cells were washed, resuspended in the same medium and incubated further. SCEs were analysed.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; purity not specified.  
13-DEC-1996 (793)

**Type:** Sister chromatid exchange assay  
**System of testing:** human peripheral lymphocytes  
**Concentration:** 10, 100, 500, 600 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 479  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide and added to the cultures for 48 hours. Diepoxybutane was used as the positive control and untreated cells acted as the negative control. Colcemid was added at the end of the treatment period and preparations made to assess SCE. In the studies using metabolic activation, cells were exposed to S9 derived from Aroclor-induced rat liver, at the same time as exposure to the highest benzene concentration tested in the study without activation. After 1 hour exposure, cells were washed and reincubated in complete medium for 24 hours. Colcemid was added and preparations made to assess SCE. Cyclophosphamide was used as the positive control in the presence of S9.

Benzene was tested at 10, 100, 500 and 600 µg/ml in the absence of S9 and at 600 µg/ml in its presence. No increase in SCE rate was noted in any of the benzene treated cultures. Similar negative results, in the absence of activation have been reported by Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978; Morimoto K. & Wolff S. Cancer Res. 40, 1189-1193, 1980.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996 (849)

**Type:** Sister chromatid exchange assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 16, 50, 160, 500, 1600, 5000 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 479  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide. Metabolic activation (S9) was derived from the liver of Aroclor-1254-induced male Sprague-Dawley rats. Cells were treated with benzene for 2 hours, bromodeoxyuridine was added and incubation continued for 24 hours. The cells were then placed in fresh medium with colcemid and incubated for 2-3 hours. When S9 was present, cells were incubated for 2 hours as above except foetal bovine serum was omitted to prevent the binding of serum proteins to short-lived, highly reactive intermediates. After the 2 hours, cells were washed, suspended in complete medium and incubated for 26 hours, colcemid being added for the final 2-3 hours. Assessment of SCEs for both treatment regimes were made. Cyclophosphamide and mitomycin C were used as positive controls in the presence and absence of S9 respectively. Benzene was tested at levels of 16, 50, 160 and 500 µg/ml in the absence of S9 and at 16, 50, 160, 500, 1600 and 5000 µg/ml in the presence of S9. No increase in the number of SCEs were found. Subsequent analyses of the data at benzene levels of 100, 250, 500, 750 and 1000 µg/ml in the absence of S9 showed an increase in SCEs. Other investigators have reported negative SCE results in this cell line when treated in the absence and presence of a metabolic activation system (Douglas G.R. et al. Prog. Mutation Res. 5, 359-366, 1985; Lane A.M. et al. *ibid.* 5, 451-455, 1985; Natarajan A.T. et al. *ibid.* 5, 433-4370, 1985), in Chinese hamster V79 cells in the absence and presence of activation (van Went G.F. *ibid.* 5, 469 477, 1985) and in rat liver cells (Priston R.A.J. & Dean B.J. *ibid.* 5, 387-395, 1985).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; >=99% pure.  
13-DEC-1996

(457) (458)

**Type:** Sister chromatid exchange assay  
**System of testing:** Chinese hamster V79 cells  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** A statistically significant increase in the number of SCEs was found in cultures treated with anti-benzene-diol-epoxide, syn-benzene-diol-epoxide, hydroquinone, catechol, 1,2,4- and 1,2,3-trihydroxybenzene.

No such effect was seen with quinone, phenol and trans-benzene-1,2-dihydrodiol. Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compounds. After 1 hour, bromodeoxyuridine was added and after 23 hours, colcemid was added. Cells were harvested and the number of SCEs scored.

**Source:** BP Chemicals Ltd LONDON (416)  
13-DEC-1996

**Type:** Unscheduled DNA synthesis  
**System of testing:** HeLa S3 cells  
**Concentration:** 0.05, 0.075, 0.1, 0.15, 0.188, 0.2, 0.375, 0.75, 1.5, 3.0 µg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 482  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene did not induce an increase in UDS in this study. The investigators thought that this absence of benzene-induced DNA-repair activity may be due to an insensitivity to the type of genotoxicity shown by benzene, an inadequate or inappropriate activation system for the production of a DNA-reactive metabolite or an inability of any reactive metabolite to live long enough to reach the DNA of the target cells. The metabolic activation system (S9 mix) was derived from the liver of Aroclor-1254-induced male Wistar rats. Cells were incubated in growth medium for 4 days, followed by growth-limiting medium for 72 hours. Hydroxyurea was added to maximise inhibition of background DNA synthesis. The cells were incubated for 1 hour and S9 mix was added, if required. Benzene was added and the mixture incubated for 2.5 hours. At the end, the cells were washed, DNA extracted and assessed. Dimethyl-sulphoxide was used as the negative, solvent control and 2 acetaminofluorene as the positive control chemical in the presence of S9 and 4-nitroquinoline-N-oxide and N-methyl-N'-nitro-N-nitroso-guanidine in the absence of S9.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure. (72)  
13-DEC-1996

**Type:** Unscheduled DNA synthesis  
**System of testing:** adult male albino rat hepatocytes  
**Concentration:** 0.078-3900 µg/ml  
**Metabolic activation:** without  
**Result:** positive

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The method of Althaus F.R. et al. Cancer Res. 42, 3010-3015, 1982 was followed. The hepatocytes were plated in normal medium which was changed after 4 hours. Benzene, dissolved in ethanol or dimethylsulphoxide, was added 18 hours before harvesting. Hydroxyurea was added 1 hour before benzene, to reduce thymidine incorporation from replicative DNA synthesis. Thymidine was added immediately after benzene. At the end of exposure, the cells were washed and assayed for UDS. Initial studies were conducted to identify the maximum effective dose. The optimal concentration for benzene was found to be 78.1 µg/ml. At this dose, a statistically significant increase ( $p < 0.05$ ) in UDS was induced. Other investigators have, however, reported negative results in rat hepatocyte UDS assays (Probst G.S. & Hill L.E. Prog. Mutation Res. 5, 381-386, 1985; Probst G.S. et al. Envir. Mutagen. 3, 11-32, 1981; Williams G.M. et al. Prog. Mutation Res. 5, 341-345, 1985).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(417)

**Type:** Gene mutation in *Saccharomyces cerevisiae*  
**System of testing:** *Saccharomyces cerevisiae* strains XV185-14C and RM52  
**Concentration:** 274.7, 549.4, 1098.8, 2197.5 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 480  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In a buffer medium at pH7.0, benzene induced histidine point mutations in the presence of S9 in XV185-14C. No tryptamine point mutations in XV185-14C were recorded. With treatment in a yeast extract peptone dextrose medium at pH6.3, arginine, histidine and tryptamine point mutations were induced in XV185-14C in the absence of S9; histidine point mutations were also present with S9 treatment. No histidine point mutations were found in RM52. A second set of investigators reported point mutations in *S. cerevisiae* strain D7 when treated with but not without S9 (Parry J.M. & Eckardt F. Prog. Mutation Res. 5, 261-269, 1985). Negative point mutation results have also been reported in strains PV-1, PV-2, PV-3 (Inge-Vecht S.G. et al. *ibid.* 5,

243-255, 1985) and D7 (Arni P. *ibid.* 5, 217-224, 1985), all tested in the presence and absence of metabolic activation. The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative control. The cultures were incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(764)

**Type:** Mitotic recombination in *Saccharomyces cerevisiae*  
**System of testing:** *Saccharomyces cerevisiae* strain D7-144  
**Concentration:** 274.7, 549.4, 1098.8, 2197.5 µg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 481  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** In a buffer medium at pH7.0, no gene conversions were found. With treatment in a yeast extract peptone dextrose medium at pH6.3, gene conversion occurred in the presence and absence of S9. A second set of investigators reported mitotic gene conversions in *S. cerevisiae* strain D7 when treated with but not without S9 (Parry T.M. & Eckardt F. *Prog. Mutation Res.* 5, 261-269, 1985). Negative mitotic gene conversion results have also been reported in strains D7 (Arni P. *et al. ibid.* 5, 217-224, 1985), JDI (Brooks T.M. *et al. ibid.* 5, 225-228, 1985), PV-2 and PV-3 (Inge-Vechtomov S.G. *et al. ibid.* 5, 243-255, 1985), all treated with and without activation.

The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative control. The culture was incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** 99% pure.  
13-DEC-1996

(764)

**Type:** Yeast Cytogenetic assay  
**System of testing:** Saccharomyces cerevisiae strains D6 and D61-M  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Aneuploidy occurred in D6 cells treated, in the absence of S9, 4 hours after inoculation into high glucose medium and in D61-M cells treated, in the absence and presence of S9, immediately after inoculation into high glucose medium. No effect was seen when this inoculation was delayed 4 hours. Cells were exposed to benzene either at the time of inoculation into high glucose medium or 4 hours after inoculation. All cultures were then grown for 18 hours on a shaker. The cells were then washed and plated on selective media to detect cell viability and mitotic aneuploidy. Benzene was dissolved in dimethylsulphoxide. Cyclophosphamide and chenodeoxycholic acid were used as the positive controls. The metabolic activation system (S9) was derived from the liver or Aroclor-induced rats.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** Lancaster synthesis, purity not specified.  
13-DEC-1996

(872)

**Type:** Sister chromatid exchange assay  
**System of testing:** Human lymphocytes  
**Concentration:** 1, 10, 25, 50, 100 & 250 ug/ml  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other: see ref.  
**Year:** 1973  
**GLP:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(786)

**Type:** Cytogenetic assay  
**System of testing:** human lymphocytes cultures  
**Concentration:** 4E-5 M and 3E-3 M  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other: see ref.  
**Year:** 1976  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(794)

**Type:** Ames test  
**System of testing:** Salmonella typhimurium strains TA97, 98, 100, 1535  
**Concentration:** 0, 0.01, 0.033, 0.1, 0.333, 1.0 mg/plate  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with OECD Guide-line 471  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Metabolic activation system was Aroclor-1254-induced S9 from male Sprague-Dawley rats and Syrian hamsters. Benzene tested at a minimum of five doses up to a toxic dose or the limit of solubility, to a maximum dose of 10 mg/plate. Triplicate plates were used. The concentration of S9 in the S9 mix was 10 or 30%. The positive controls were 9-aminoacridine, 2-aminoanthracene, 4-nitro-O-phenylenediamine and sodium azide.  
Reliability: 1 (valid)  
Comparable to guideline study TA 97 instead of TA 1537 used  
results: Benzene showed no evidence of mutagenicity in any of the strains when tested in the absence of metabolic activation or in the presence of 10 or 30% hamster or rat liver S9. Several other investigators have also reported negative findings in S. typhimurium reverse mutation assays using several tester strains. Some of these include Cotruvo J.A. et al. Ann. N.Y. Acad. Sci. 298, 124-140, 1977; Florin I. et al. Toxicology 15, 219-232, 1980; Matsushima T. et al. Prog. Mutation Res. 5, 181-186, 1985; Shimizu M. et al. Mutation Res. 116, 217-238, 1983.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99% pure.

06-JAN-1997

(1305)

**Type:** other: Salmonella typhimurium forward mutation assay  
**System of testing:** Salmonella typhimurium strain TM667  
**Concentration:** <=13mM (1014 mg/l)  
**Metabolic activation:** with  
**Result:** negative

**Method:** other  
**Year:** 1978  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
no concurrent negative control, no data about cytotoxicity  
The mutation assay was carried out according to Skopek T.R. et al. Proc. natn. Acad. Sci. U.S.A. 75, 410-414 & 4470-4473, 1978. Bacteria exposed for 2 hr in the presence of 10% of a postmitochondrial supernatant, prepared as a 25% liver homogenate of phenobarbital- or Aroclor-pretreated

male Sprague-Dawley rats. Exposure followed by 2 d postexposure incubation period. Resistance to 8-azaguanine was used as the genetic marker.

results: No evidence of mutagenicity was seen when benzene was tested up to a concentration of 13 mM in this strain in the presence of metabolic activation systems. Similar results were reported by Seixas, G.M. et al., Mutat. Res. 102, 201-212 (1982, Reliability: 2 (valid with restriction))

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Supplied by Aldrich Chemical Co., Milwaukee, Wisconsin; purity unspecified.

06-JAN-1997

(591)

**Type:** other: Ames test (preincubation assay)

**System of testing:** Salmonella typhimurium strains TA98, 100, 104, 1535

**Concentration:** 3-1000ppm (converts to 0.009- 3.26 mg/l)

**Metabolic activation:** with and without

**Result:** positive

**Method:** other

**GLP:** no data

**Test substance:** no data

**Remark:** Reliability: 2 (valid with restriction)

No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

no positive control, TA1537 not tested; no data about cytotoxicity, test substance, standard deviation

results: Benzene was found to be mutagenic in the presence but not the absence of S9. Strain TA1535 was the most responsive. A benzene concentration as low as 0.03 mg/l caused a two-fold increase in the number of mutants above the control. In this same strain, the metabolites trans-benzene-1,2-dihydrodiol in the presence of S9 and anti-benzene-diol-epoxide and syn-benzene-diol-epoxide in the absence of S9 induced mutations. No other metabolite, including catechol to which trans-benzene-1,2-dihydrodiol is converted by cytosolic dihydrodiol dehydrogenase, gave a positive result in strain TA1535. Mutagenic responses, some of them weak, were noted in other strains treated with 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, catechol, quinone, hydroquinone, syn-benzene-diol-epoxide and anti-benzene-diol-epoxide.

Tests were conducted on benzene and its following metabolites: benzene oxide, phenol, hydroquinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxy-biphenyl, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-tri-hydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. S. typhimurium was exposed to benzene vapour in desiccators to allow for longer exposure periods. S9 mix derived from rat and mouse liver homogenate was used as the activation system. Metabolites also tested with strains TA97 and 102.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(416)

**Type:** HGPRT assay  
**System of testing:** Chinese hamster V79 cells  
**Concentration:** see remarks  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 3 (not valid)  
Documentation insufficient for assessment  
no data about metabolic activation, concentration used, test substance, survival, positive control, number of tests  
results: Mutations to 6-thioguanine resistance were induced by quinone and hydroquinone (potent inducer), anti-benzene-diol-epoxide, catechol, trans-benzene-1,2-dihydrodiol and 1,2,4-trihydroxybenzene. The cytotoxicity and potency of the mutagenic effect varied between the chemicals. Phenol, the dihydroxybiphenyls and duroquinone showed no mutagenic effect. Anti-benzene-diol epoxide induced a significant but weak mutation to ouabain resistance.  
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxybiphenyl, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone and anti-benzene-diol-epoxide were also included. Compounds were tested up to their cytotoxic limits. Cells were mixed with the test compound for 24 hours and mutants were determined after an expression period of 6 days.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(416)

**Type:** HGPRT assay  
**System of testing:** Chinese hamster V79 cells  
**Concentration:** 0.1, 0.3, 1.0, 2.0 mg/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Preliminary cytotoxicity studies were carried out using this same cell-line, with and without 5% foetal calf serum. Benzene was added over a concentration range of 5 'g/ml to 10 mg/ml in the presence and absence of a metabolic activation system (S9 mix). Cells were examined microscopically at intervals up to 4 hr for evidence of damage. Based on the results of this study, a dose range was chosen for use in assays for inhibition of colony-forming ability. This was conducted in the presence and absence of serum and the presence and absence of S9 mix for periods of up to 4 hr. For the mutagenicity assay, the

cells were exposed to benzene for 1 hr in the absence of serum and the presence and absence of S9 mix. Cyclophosphamide and ethylmethanesulphonate were used as positive controls, in the presence and absence of S9 mix respectively. The concentrations of benzene tested included at least one dose that induced measurable cytotoxicity and one higher dose. The S9 mix used was derived from livers of male albino rats treated with phenobarbital and beta-naphthoflavone.

Reliability: 1 (valid)

Comparable to guideline study

results: The maximum non-toxic benzene dose in the cytotoxicity assay was 0.31 mg/ml when tested for 4 hr in the presence of serum and the presence and absence of S9 or in the absence of serum and the presence of S9. When tested without S9, in the absence of serum, the maximum non-toxic dose was 0.63 mg/ml. In the colony forming assay, the highest non-toxic dose when tested with and without serum and with and without S9 mix was 0.003 mg/ml and the concentration which killed 50% of the cells in the presence of serum and the absence and presence of S9 mix was 1 mg/ml and >1 mg/ml in the absence of serum. In the mutagenicity assay, the mutation frequency was evaluated 5 and 8 days after exposure. No mutagenic activity was found.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(368)

**Type:** HGPRT assay  
**System of testing:** Chinese hamster V79 cells  
**Concentration:** 1, 10, 50, 100, 500, 1000 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 476

**Year:** 1984

**GLP:** no data

**Test substance:** other TS

**Remark:** A preliminary cytotoxicity study was carried out using this same cell line treated with 1, 10, 50, 100 and 500 ug/ml for 3 hours. The treated cells were then rinsed with Hanks' solution and incubated in normal medium for a further 7 days before the colony-forming activity was evaluated. The mutagenic activity was determined by the 'replating' method. Cells were treated for 3 hours with benzene, rinsed with Hanks' solution and then incubated in normal medium for 6 days. They were then treated with trypsin, replated in medium containing 6-thioguanine and incubated for 14 days. The number of 6-thioguanine-resistant colonies was scored. The assay was conducted in the absence and presence of a metabolic activation system (S9 mix) which had been obtained from male Sprague-Dawley rats treated with an intraperitoneal injection of phenobarbital and 5,6-benzoflavone. Benzene was dissolved in dimethylsulphoxide before use and diluted with Hanks' solution to obtain the appropriate concentration for

treatment of cells. The vehicle alone was tested as the 0 ug benzene/ml level and ethyl-methanesulphonate was the positive control chemical.

Reliability: 1 (valid)

Comparable to guideline study

results: Cells treated with benzene at 100 and 500 ug/ml had slightly decreased colony-forming activities, showing slight cytotoxicity. A slight mutagenic effect was noted at 50 and 100 ug/ml, tested in the absence of S9 mix. No mutations were noted at any concentration in the presence of S9 mix.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(648)

**Type:** Mouse lymphoma assay

**System of testing:** L5178Y mouse lymphoma cells

**Concentration:** 66, 88, 132, 176, 352, 528 ug/ml

**Metabolic activation:** with and without

**Result:** negative

**Method:** other: in compliance with OECD Guide-line 476

**Year:** 1984

**GLP:** no data

**Test substance:** other TS

**Remark:** Preliminary cytotoxicity tests were conducted in the presence and absence of metabolic activation. The metabolic activation systems were liver enzymes from Aroclor-1254-induced male SPF Wistar rats (S9 mix), primary chick embryo hepatocytes, and Syrian hamster embryo cells. The results of this test were used to select the concentrations to be used in the mutagenicity assay, with the lowest concentration showing no toxicity and the highest giving a survival of about 15% immediately after treatment. In the mutagenicity test, cells were treated for 2 hours in the absence or presence of S9 and for 20 hours in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. After treatment, the cells were seeded for survival or propagated for several days in standard medium to allow for the expression of induced mutants. Cloning efficiency and selection of mutants were made and mutant frequency assessed. Benzene was dissolved or diluted to the appropriate test concentration in dimethylsulphoxide and this solvent was included in each experiment at 1% as the negative control. Ethylnitrosourea, diethylnitrosamine and benzo(a)pyrene acted as the positive controls in the experiments with no activation, activated with S9 and activated with Syrian hamster-embryo cells and primary chick-embryo hepatocytes respectively.

Reliability: 1 (valid)

Comparable to guideline study

no data about 2nd independent experiment

results: Cells treated for 2 hours in the absence and presence of S9 showed 66.6 and 1% survival respectively at a benzene concentration of 352 ug/ml. The cells were completely killed at concentrations of 704 and 528 ug/ml when treated in the absence and presence of S9 respectively.

In the mutagenicity assays, benzene was tested at concentrations of 88, 176, 352 and 528 ug/ml in the absence of any activation; 88, 176 and 352 ug/ml in the presence of S9 and at 66 and 132 ug/ml in the presence of Syrian hamster-embryo cells and primary chick-embryo hepatocytes. No evidence of mutagenicity at the thymidine kinase locus or the hypoxanthine guanine phosphoribosyltransferase locus was seen in any of the systems. Similar negative findings at the thymidine kinase locus have been reported by Amacher D.E. & Turner G.N. Prog. Mutation Res. 5, 487-496, 1985; Myhr B et al. ibid. 5, 555-568, 1985; Oberly T.J. et al. Mutation Res. 125, 291-306, 1984.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997

(624)

**Type:** Mouse lymphoma assay  
**System of testing:** L5178Y mouse lymphoma cells  
**Concentration:** 500-1200 ug/ml and 100-800 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was tested dissolved in dimethylsulphoxide. The metabolic activation system (S9) was prepared from the livers of male Fischer 344 rats pretreated with Aroclor-1254. Preliminary toxicity studies previously conducted at the same laboratory were used to determine the concentrations to be used in the mutagenicity assay. The highest dose selected was that concentration causing a 50-90% inhibition of growth in the preliminary study. In the mutagenicity study, cells were treated with benzene for 4 hours, washed, resuspended in normal medium and incubated for a further 48 hours. Mutant frequency at the thymidine kinase locus was then evaluated after a further 12-day incubation period. This procedure was repeated with the addition of S9. Dimethylsulphoxide (1%) was used as the negative solvent control and ethylmethanesulphonate and 3-methyl-cholanthrene as the positive controls in the nonactivated and activated tests respectively.  
Reliability: 1 (valid)  
Comparable to guideline study  
no data about 2nd independent experiment  
results: Based on former toxicity data, concentrations of 500-1200 ug/ml and 100-800 ug/ml were selected for the nonactivated and activated tests respectively. In the absence of S9, 1000 ug/ml killed all the test culture and 49% survival was noted at 600 ug/ml. In the presence of S9, 8% survival was seen at a benzene concentration of 800 ug/ml and 45% survival occurred at 100 ug/ml. In the mutagenicity test, benzene was found not to be mutagenic in the absence of S9. In the presence of S9, cultures exposed to 100, 300, 400 and 500 ug/ml showed 2-3-fold increases in mutant

frequency over the background. Survival in these cultures exceeded 10% and benzene was, therefore, judged to be mutagenic in the presence of activation.

A similar positive result was reported by Styles J.A. et al.

Prog. Mutation Res. 5, 587-596, 1985: In the presence of S9, benzene produced an unequivocal positive result at the Na<sup>+</sup>/K<sup>+</sup> ATPase locus (selected by resistance to ouabain) and a weak result at the thymidine kinase locus (no such effect was found in the absence of S9 but this was thought to be due to excessive toxicity of benzene).

Matthews, E.J. et al., Prog. Mutat. Res. 5, 639-650 (1985), reported also increased mutation frequency to ouabain resistance at the Na<sup>+</sup>/K<sup>+</sup> ATPase locus.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(850)

**Type:** Mouse lymphoma assay  
**System of testing:** L5178Y mouse lymphoma cells  
**Concentration:** 12.5-200 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 476

**Year:** 1984

**GLP:** no data

**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about mean value and deviation, 2 plates per dose,  
no 2nd independent experiment  
results: From the preliminary study, a dose range of  
12.5-200 ug/ml was determined. The mutant frequency was  
statistically significantly increased in the 6-thioguanine  
test in the absence of S9. No increased mutations to ouabain  
resistance were detected.

The method of Cole J. et al. Mutation Res. 41, 377-386,  
1976 was followed. Benzene was dissolved in  
dimethylsulphoxide and diluted to obtain the required  
concentration. Cells were treated in the presence and  
absence of S9 mix (derived from Aroclor-1254-induced rats)  
for 2 hours. Survival was determined initially at the  
various concentrations tested without S9. In the  
mutagenicity assay, after treatment, the cells were cultured  
for 48 hours, mixed with ouabain and incubated for 2 weeks  
or cultured for 7 days, mixed with 6-thioguanine and  
incubated for 2 weeks. Benzo(a)pyrene and  
4-nitroquinoline-1-oxide were the positive control chemicals  
for the assays conducted with and without S9 respectively.  
Dimethylsulphoxide was the negative solvent control.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade.

06-JAN-1997

(394)

**Type:** Cytogenetic assay  
**System of testing:** human lymphocytes  
**Concentration:** 9, 44, 88 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 473  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about mitogen, harvest time, mitotic index  
results: In the cytotoxicity study, a 50-80% reduction in mitosis was seen in cultures treated with 88 ug/ml when compared with the negative control. The mutagenicity test was conducted at benzene concentrations of 9, 44 and 88 ug/ml. A dose-related increase in chromosomal aberrations was noted in both the male and female donated lymphocytes treated in the absence and presence of S9. The incidence of all aberrations, excluding chromatid gaps, was statistically significantly increased over the negative controls, with all treatment regimes; this being more pronounced in the males than females (not significant in females at the low dose +/- S9 and at mid dose -S9).  
A second set of investigators reported chromosomal aberrations in human lymphocytes treated in the absence of metabolic activation (Morimoto K., Jap. J. ind. Hlth 18, 23-34, 1976), however, negative results in the absence of activation have also been reported (Gerner-Smidt P. & Friedrich U., Mutation Res. 58, 313-316, 1978).  
The method of Richardson C.R. & Wildgoose J. Mutation Res. 100, 287-293, 1982 was followed. Benzene was dissolved in dimethylsulphoxide before use and dimethyl-sulphoxide was used to treat the negative control cultures. Metabolic activation system was derived from the liver of Aroclor-1254-induced rats. Blood was withdrawn from a donor of each sex who had a previously established low incidence of chromosomal damage. Preliminary cytotoxicity tests were performed in the absence and presence of S9. In the chromosome aberration test, the lymphocyte cultures were treated with benzene, 44 hours after initiation for 3 hours. When activation was used, benzene and the relevant controls were incubated for 30 min with S9 before being added to the blood cultures. The cultures exposed to benzene were agitated throughout the exposure period to maintain homogeneity of the chemical through the culture. Mitomycin C was used as the positive control substance in the absence of S9 and cyclophosphamide in the presence of S9. Lymphocytes were treated with colchicine before analysis.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997 (526)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 200, 600, 1800 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 473  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 1 (valid)  
Comparable to guideline study results:  
In cultures fixed after 3 hours, significant increases in the frequency of chromosomal aberrations were seen in the absence and presence of S9 at 1800 and 600 ug/ml respectively. No such effects were found after 12 hours and after 18 hours, significant increases were observed at 200 and 600 ug/ml in the presence of S9. A second study conducted on a Chinese hamster lung fibroblast cell line also showed benzene-induced chromosome aberrations when tested in the presence but not the absence of S9 (Ishidate M. Jr & Sofuni T., Prog. Mutation Res. 5, 427-432, 1985). The metabolic activation system (S9 mix) was prepared from the livers of phenobarbital- and beta-naphthoflavone-induced male Sprague-Dawley/CD rats. Benzene was dissolved in dimethylsulphoxide to achieve the required concentration. The negative control consisted of both untreated and solvent treated cultures. Cyclophosphamide and aflatoxin B1 were used as the positive controls. Benzene was tested at concentrations of 200, 600 and 1800 ug/ml, the top dose giving approximately 50% inhibition of the mitotic index. Cells were treated for 3 hours, the medium aspirated and cultures washed. Some cultures were fixed while others were resuspended in fresh medium and incubated for 6 or 12 hours. Colchicine was added to these cultures and 3 hours later they were fixed.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997

(867)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** not specified  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about mitotic index, single harvest time  
results:

Benzene failed to induce chromosome aberrations when in either the absence or presence of S9. Similar negative findings have also been reported in this same cell line by Natarajan A.T. et al. Prog. Mutation Res. 5, 433-437, 1985; in a Chinese hamster fibroblast cell line in the absence of a metabolic activation system by Danford N. *ibid.* 5, 397-411, 1985; and in Wistar rat liver cells in the absence of metabolic activation by Priston R.A.J. & Dean B.J. *ibid.* 5, 387-395, 1985.

The method of Galloway S.M. et al. *Envir. Mutagen.* 7, 1-51, 1985 was followed. Benzene was dissolved in dimethylsulphoxide to the required test concentration. The metabolic activation system (S9) was prepared from Aroclor-1254-induced male Sprague-Dawley rats. The dose levels used in the study were selected based on the toxicity of benzene observed in concurrently run sister-chromatid exchange studies. In the absence of S9 treatment, cells were exposed to benzene for 10 hours. Colcemid was added 2-3 hours prior to cell harvest. In the presence of S9, cells were treated with benzene and S9 for 2 hours. The cells were then washed, returned to complete medium and incubated for 11 hours, colcemid being added 2 hours before harvest.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; >=99% pure.  
06-JAN-1997

(457) (458)

**Type:** other: DNA damage assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 1.33 mg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Metabolic activation system was the S9 fraction from Aroclor-1254-induced rat liver. Benzene was dissolved in dimethylsulphoxide. The cells were incubated in the presence of benzene for 30 min, 1 or 2 hours. Metabolic activation was only added in the 1 hour study. Survival of the cells was also checked. The single-strand DNA breaks were detected by high-salt alkaline sucrose-gradient sedimentation. Untreated cells acted as the negative control and ethylmethanesulphonate and diethyl-nitrosamine treated cells as the positive controls.  
Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
single concentration, no solvent control, no data about 2nd independent experiment, no negative control with S9  
results:  
After a treatment time of 1 hour, 20.9 and 33.0% survival was seen in the cells treated without and with S9 respectively. Single-strand breaks were greatly increased

after 30 min, 1 and 2 hours treatment in the absence of S9; the increase being time-dependent. No such effect was noted in the presence of S9.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(655)

**Type:** other: DNA damage assay

**System of testing:** Chinese hamster ovary cells

**Concentration:** 0.39-7.81 mg/ml

**Metabolic activation:** with and without

**Result:** negative

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide before addition to the cells. Metabolic activation system was derived from the liver of Aroclor-1254 induced Sprague-Dawley rats (S9 mix). Cells were treated for 1 hour with benzene, washed and subjected to alkaline sucrose sedimentation analysis in order to assess the DNA single-strand breaks. Prior to testing benzene, its cytotoxicity was determined by examining inhibition of cell growth.

Reliability: 2 (valid with restriction)

No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

no positive control, single exposure time, no data on negative control and 2nd independent experiment results:

The cytotoxicity threshold, that is, the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 'g/ml when tested in the presence and absence of S9. In the mutagenicity study, benzene was tested at levels of 0.39-7.81 mg/ml. No single-strand breaks were found at levels up to 2.34 mg/ml tested with or without S9. Above this, single-strand breaks increased but this was thought to be due to the toxic effects of benzene. Other investigators have reported similar negative results using the alkaline elution technique in Chinese hamster V79 cells in the presence and absence of metabolic activation (Swenberg J.A. et al. Biochem. biophys. Res. Commun. 72, 732-738, 1976) and rat hepatocytes in the absence of activation (Bradley M.O. Prog. Mutation Res. 5, 353-357, 1985; Sina J.F. et al. Mutation Res. 113, 357-391, 1983).

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; purity unspecified.

06-JAN-1997

(305)

**Type:** other: cell transformation assay  
**System of testing:** Balb/c-3T3 mouse embryo cells  
**Concentration:** 0.5, 1.67, 2.0, 3.33, 4.0, 5.0 mg/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with Directive 87/302/EEC, part B, p. 73  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** A cytotoxicity study was initially carried out to determine the benzene doses to be tested in the cell transformation assay. Doses causing 10-100% survival of the cells were used. In the nonactivation assay cells were treated with benzene for 72 hours, washed and incubated for a further 24 days. Transformed foci were scored microscopically. For metabolic activation primary rat-liver cells were prepared from Fischer-344 rats and lethally X-irradiated. These feeder cells were added to the mouse embryo cells for 3 hours before treatment with benzene for 48 hours. The cultures were then treated with 12-O-tetra-decenoylphorbol-13-acetate biweekly for 3 weeks, starting 1-2 days after benzene treatment. Dimethylnitrosamine and 3-methylcholanthrene were used as the positive controls in the tests conducted with and without the addition of the rat liver cells respectively. Untreated cells were the negative controls. The tests were conducted in closed tissue culture flasks.  
Reliability: 1 (valid)  
Comparable to guideline study  
no data about 2nd independent experiments  
results:  
In the cytotoxicity test, 59% survival was seen at doses of 3.0 and 3.33 mg/ml in the absence and presence of activation respectively. All cells were killed at 6.0 mg/ml in the absence of activation and 0.3% survival was found at 5.0 mg/ml with activation. In the transformation assay, doses of 0.5, 2.0 and 4.0 mg/ml and 1.67, 3.33 and 5.0 mg/ml were tested without and with activation respectively. In the absence of activation, no transformation activity was found. In the presence of activation, statistically significant effects were noted at all dose levels, the increases in the numbers of transformed cell foci being dose-related. A second set of investigators also reported a lack of transforming activity in this cell line when benzene was tested in the absence of activation. The test was not conducted, in this case, with added activation (Rundell J.O. et al. *Envir. Sci. Res.* 27, 309-324, 1983).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; purity 99%.  
06-JAN-1997 (748)

**Type:** other: cell transformation assay  
**System of testing:** C3H/10T« mouse embryonic fibroblast cells  
**Concentration:** 50, 80, 100, 160, 200, 320 ug/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with Directive 87/302/EEC, part B, p. 73  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved and diluted in dimethylsulphoxide before use. The metabolic activation system (S9) was obtained from the liver of Aroclor-1254-treated male rats. A dose-ranging study was conducted to determine the doses to be used in a preliminary cytotoxicity test. The latter was conducted to establish the range of concentrations to be used in the cell transformation assay. In the transformation assay, the cells were exposed to benzene for 24 hours in the presence or absence of S9 mix. One of the treated cultures/dose was used to assess cell survival in the study. In the remaining cultures, the medium was changed twice weekly until the cells reached confluence and weekly thereafter. After an 8-week incubation period, the cultures were stained and assessed for cell transformation. Dimethylsulphoxide was used as the negative control chemical and cyclophosphamide as the positive control in the presence of S9. In the absence of the activation mix, 3-methylcholanthrene acted as the positive control.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data on 2nd independent experiment, cytotoxicity not specified, no data on statistical evaluation, no data how test results judged to be positive  
results:  
Based on the preliminary toxicity tests, benzene was tested at concentrations of 50, 100 and 200 ug/ml in the absence and 80, 160 and 320 ug/ml in the presence of S9. No transformed foci were identified at any benzene concentration in the presence or absence of S9. A negative or marginally significant positive result in this cell line in the absence of activation was reported by Nesnow S. et al. Prog. Mutation Res. 5, 659-664, 1985.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997 (669)

**Type:** other: cell transformation assay  
**System of testing:** Syrian hamster embryo (SHE) cells  
**Concentration:** 20-500 ug/ml  
**Metabolic activation:** with  
**Result:** positive

**Method:** other: in compliance with Directive 87/302/EEC, part B, p. 73  
**Year:** 1988  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Lethally X-irradiated SHE cells were seeded and 24 hours later target cells were added. After 24 hours, benzene was added and the cells were incubated for 6 days. The medium was then removed, the cells rinsed and benzene added for a further 24 hours. The cells were then washed and transformation activity assessed. Benzene was dissolved in dimethylsulphoxide. Benzo(a)pyrene was used as the positive control.

Reliability: 1 (valid)

Comparable to guideline study results:

Positive results were obtained at concentrations of 20 ug/ml and above. However, at concentrations higher than 300 ug/ml, the transformation frequency decreased. Similar positive results in the absence of activation have been reported in this cell line by Barrett J.C. & Lamb P.W. Prog. Mutation Res. 5, 623-628, 1985. Amacher and Zelljadt reported a low transformation frequency in SHE cells when tested in the absence of metabolic activation (Amacher D.E. & Zelljadt I. Carcinogenesis 4, 291-295, 1983) and a negative result has been reported in the SHE cell-transformation enhancement assay using SA7 viruses, again in the absence of activation (Hatch G.G. & Anderson T.M. Prog. Mutation Res. 5, 629-638, 1985).

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(997)

**Type:** other: micronucleus assay in vitro  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 7.81-7810 ug/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide before addition to the culture medium. Aroclor-1254-induced Sprague-Dawley rat-liver S9 was used as the metabolic activation system. A preliminary cytotoxicity test was conducted in which cells were incubated with a range of benzene concentrations with and without S9 for 24 hours. From this test, the concentrations to be used in the micronucleus assay were selected. In the micronucleus assay, cells were treated for

1 hour, washed and incubated in normal medium for 24 hours. The micronucleus frequency was then assessed. Historic dimethylsulphoxide control data were used as the background readings.

Reliability: 3 (not valid)

Significant methodological deficiencies  
no positive control, no statistical evaluation, no data about mitotic index and 2nd independent experiment, insufficient presentation of results

results:

In the preliminary study, the cytotoxicity threshold, that is the lower limit of cytotoxicity as determined by visible inhibition of cell growth, was found to be 0.781 ug/ml when in both the absence and presence of S9. In the micronucleus assay, no evidence of mutagenicity was seen with and without S9 at concentrations of 7.81 mg/ml to 7.81 ug/ml.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(305)

**Type:** other: micronucleus assay in vitro

**System of testing:** Chinese hamster V79 cells

**Metabolic activation:** no data

**Result:** positive

**Method:** other

**GLP:** no data

**Test substance:** no data

**Remark:** Reliability: 3 (not valid)

Documentation insufficient for assessment  
no data about metabolic activation, number of experiments/dose, survival, positive control, test substance results:

Induction of micronuclei was reported in cells treated with anti-benzene-diol-epoxide, quinone, hydroquinone, catechol, phenol, 1,2,4-trihydroxybenzene, 1,2,3-trihydroxybenzene and duroquinone. The potency of this effect varied between the chemicals. No such effect was seen with syn-benzene-diol-epoxide and trans-benzene-1,2-dihydrodiol. Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Duroquinone, anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compound for 24 hours. They were then harvested and scored for micronuclei. Compounds were tested up to their cytotoxic limits.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(416)

**Type:** Sister chromatid exchange assay  
**System of testing:** human peripheral lymphocytes  
**Concentration:** 16, 78, 391 mg/l  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 479  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** An increased number of SCEs were found in cultures treated in the presence of 10% S9 mix. In the absence of S9 and at S9 concentrations of 1 or 90% no increase in the frequency of SCEs was noted. 10% S9 mix is thought to be the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. When the cells were exposed to benzene concentrations of  $2 \times 10^{-4}$ ,  $1 \times 10^{-3}$  and  $5 \times 10^{-3}M$  (approximately 16, 78 and 391 mg/l), a dose-related increase in SCEs was seen when the appropriate activation concentration was used. S9 mix at 10-30% converted benzene into active forms that were cytotoxic and delayed cell turnover times. Further examination of this suggested that the metabolites responsible for cell division delay may be different from those which induce SCE. The addition of GSH to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S9 mix. The addition of GSH also completely prevented the induction of SCEs by catechol and hydroquinone, two major phenolic metabolites of benzene and potent inducers of SCEs.

Blood was obtained from healthy adult men. Benzene was dissolved in serum-free culture medium. Cells were suspended in the serum-free culture medium containing benzene and the metabolic activation system (S9 mix derived from rat liver) and incubated for 2 hours. The flask was agitated to ensure even distribution of active metabolites among the cells. After incubation, the cells were washed, resuspended in the same medium and incubated further. SCEs were analysed.

Reliability: 1 (valid)  
Comparable to guideline study  
no positive control

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; purity not specified.  
06-JAN-1997

(793)

**Type:** Sister chromatid exchange assay  
**System of testing:** human peripheral lymphocytes  
**Concentration:** 10, 100, 500, 600 ug/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with OECD Guide-line 479  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide and added to the cultures for 48 hours. Diepoxybutane was used as the positive control and untreated cells acted as the negative control. Colcemid was added at the end of the treatment period and preparations made to assess SCE. In the studies using metabolic activation, cells were exposed to S9 derived from Aroclor-induced rat liver, at the same time as exposure to the highest benzene concentration tested in the study without activation. After 1 hour exposure, cells were washed and reincubated in complete medium for 24 hours. Colcemid was added and preparations made to assess SCE. Cyclophosphamide was used as the positive control in the presence of S9.

Reliability: 1 (valid)

Comparable to guideline study results:

Benzene was tested at 10, 100, 500 and 600 ug/ml in the absence of S9 and at 600 ug/ml in its presence. No increase in SCE rate was noted in any of the benzene treated cultures. Similar negative results, in the absence of activation have been reported by Gerner-Smidt P. & Friedrich U. Mutation Res. 58, 313-316, 1978; Morimoto K. & Wolff S. Cancer Res. 40, 1189-1193, 1980.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(849)

**Type:** Sister chromatid exchange assay  
**System of testing:** Chinese hamster ovary cells  
**Concentration:** 16, 50, 160, 500, 1600, 5000 ug/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with OECD Guide-line 479  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in dimethylsulphoxide. Metabolic activation (S9) was derived from the liver of Aroclor-1254-induced male Sprague-Dawley rats. Cells were treated with benzene for 2 hours, bromodeoxyuridine was added and incubation continued for 24 hours. The cells were then placed in fresh medium with colcemid and incubated for 2-3 hours. When S9 was present, cells were incubated for 2 hours as above except foetal bovine serum was omitted to

prevent the binding of serum proteins to short-lived, highly reactive intermediates. After the 2 hours, cells were washed, suspended in complete medium and incubated for 26 hours, colcemid being added for the final 2-3 hours. Assessment of SCEs for both treatment regimes were made. Cyclophosphamide and mitomycin C were used as positive controls in the presence and absence of S9 respectively. Reliability: 1 (valid)

Comparable to guideline study  
results:

Benzene was tested at levels of 16, 50, 160 and 500 ug/ml in the absence of S9 and at 16, 50, 160, 500, 1600 and 5000 ug/ml in the presence of S9. No increase in the number of SCEs were found. Subsequent analyses of the data at benzene levels of 100, 250, 500, 750 and 1000 ug/ml in the absence of S9 showed an increase in SCEs (but judged by the authors as negative result).

Other investigators have reported negative SCE results in this cell line when treated in the absence and presence of a metabolic activation system (Douglas G.R. et al. Prog. Mutation Res. 5, 359-366, 1985; Lane A.M. et al. ibid. 5, 451-455, 1985; Natarajan A.T. et al. ibid. 5, 433-4370, 1985), in Chinese hamster V79 cells in the absence and presence of activation (van Went G.F. ibid. 5, 469 477, 1985) and in rat liver cells (Priston R.A.J. & Dean B.J. ibid. 5, 387-395, 1985).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; >=99% pure.  
06-JAN-1997

(457) (458)

**Type:** Sister chromatid exchange assay  
**System of testing:** Chinese hamster V79 cells  
**Metabolic activation:** no data  
**Result:** positive

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 3 (not valid)  
Documentation insufficient for assessment  
no data about metabolic activation, 2nd independent experiment, positive control, partly test conditions, test substance  
results:  
A statistically significant increase in the number of SCEs was found in cultures treated with anti-benzene-diol-epoxide, syn-benzene-diol-epoxide, hydroquinone, catechol, 1,2,4- and 1,2,3-trihydroxybenzene. No such effect was seen with quinone, phenol and trans-benzene-1,2-dihydrodiol.  
Tests were conducted using the following benzene metabolites: phenol, hydroquinone, quinone, trans-benzene-1,2-dihydrodiol, catechol and 1,2,4-trihydroxybenzene. Anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. Cells were mixed with the test compounds.

After 1 hour, bromodeoxyuridine was added and after 23 hours, colcemid was added. Cells were harvested and the number of SCEs scored.

**Source:** Deutsche Shell Chemie GmbH Eschborn (416)  
06-JAN-1997

**Type:** Unscheduled DNA synthesis  
**System of testing:** HeLa S3 cells  
**Concentration:** 0.05, 0.075, 0.1, 0.15, 0.188, 0.2, 0.375, 0.75, 1.5, 3.0 ug/ml  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** other: in compliance with OECD Guide-line 482  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 1 (valid)  
Comparable to guideline study results:  
Benzene did not induce an increase in UDS in this study. The investigators thought that this absence of benzene-induced DNA-repair activity may be due to an insensitivity to the type of genotoxicity shown by benzene, an inadequate or inappropriate activation system for the production of a DNA-reactive metabolite or an inability of any reactive metabolite to live long enough to reach the DNA of the target cells.  
The metabolic activation system (S9 mix) was derived from the liver of Aroclor-1254-induced male Wistar rats. Cells were incubated in growth medium for 4 days, followed by growth-limiting medium for 72 hours. Hydroxyurea was added to maximise inhibition of background DNA synthesis. The cells were incubated for 1 hour and S9 mix was added, if required. Benzene was added and the mixture incubated for 2.5 hours. At the end, the cells were washed, DNA extracted and assessed. Dimethyl-sulphoxide was used as the negative, solvent control and 2 acetaminofluorene as the positive control chemical in the presence of S9 and 4-nitroquinoline-N-oxide and N-methyl-N'-nitro-N-nitroso-guanidine in the absence of S9.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure. (72)  
06-JAN-1997

**Type:** Unscheduled DNA synthesis  
**System of testing:** adult male albino rat hepatocytes  
**Concentration:** 0.078-3900 ug/ml  
**Metabolic activation:** without  
**Result:** positive

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, no independent 2nd test, no data about  
cytotoxicity  
results:

The optimal concentration for benzene was found to be 78.1 ug/ml. At this dose, a statistically significant increase ( $p < 0.05$ ) in UDS was induced. Other investigators have, however, reported negative results in rat hepatocyte UDS assays (Probst G.S. & Hill L.E. Prog. Mutation Res. 5, 381-386, 1985; Probst G.S. et al. Envir. Mutagen. 3, 11-32, 1981; Williams G.M. et al. Prog. Mutation Res. 5, 341-345, 1985).

The method of Althaus F.R. et al. Cancer Res. 42, 3010-3015, 1982 was followed. The hepatocytes were plated in normal medium which was changed after 4 hours. Benzene, dissolved in ethanol or dimethylsulphoxide, was added 18 hours before harvesting. Hydroxyurea was added 1 hour before benzene, to reduce thymidine incorporation from replicative DNA synthesis. Thymidine was added immediately after benzene. At the end of exposure, the cells were washed and assayed for UDS. Initial studies were conducted to identify the maximum effective dose.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(417)

**Type:** Gene mutation in *Saccharomyces cerevisiae*  
**System of testing:** *Saccharomyces cerevisiae* strains XV185-14C and RM52  
**Concentration:** 274.7, 549.4, 1098.8, 2197.5 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 480  
**Year:** 1986  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about 2nd independent experiment, 4 instead of 5  
recommended concentrations  
results:  
In a buffer medium at pH7.0, benzene induced histidine point mutations in the presence of S9 in XV185-14C. No tryptamine point mutations in XV185-14C were recorded. With treatment

in a yeast extract peptone dextrose medium at pH6.3, arginine, histidine and tryptamine point mutations were induced in XV185-14C in the absence of S9; histidine point mutations were also present with S9 treatment. No histidine point mutations were found in RM52. A second set of investigators reported point mutations in *S. cerevisiae* strain D7 when treated with but not without S9 (Parry J.M. & Eckardt F. Prog. Mutation Res. 5, 261-269, 1985). Negative point mutation results have also been reported in strains PV-1, PV-2, PV-3 (Inge-Vechtsov S.G. et al. ibid. 5, 243-255, 1985) and D7 (Arni P. ibid. 5, 217-224, 1985), all tested in the presence and absence of metabolic activation. The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative solvent control. The cultures were incubated with benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Laboratory reagent grade; 99% pure.

06-JAN-1997

(764)

**Type:** Mitotic recombination in *Saccharomyces cerevisiae*

**System of testing:** *Saccharomyces cerevisiae* strain D7-144

**Concentration:** 274.7, 549.4, 1098.8, 2197.5 ug/ml

**Metabolic activation:** with and without

**Result:** positive

**Method:** other: in compliance with OECD Guide-line 481

**Year:** 1986

**GLP:** no data

**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions  
no data about 2nd independent experiment, 4 instead of 5  
recommended concentrations tested  
results:

In a buffer medium at pH7.0, no gene conversions were found. With treatment in a yeast extract peptone dextrose medium at pH6.3, gene conversion occurred in the presence and absence of S9. A second set of investigators reported mitotic gene conversions in *S. cerevisiae* strain D7 when treated with but not without S9 (Parry T.M. & Eckardt F. Prog. Mutation Res. 5, 261-269, 1985). Negative mitotic gene conversion results have also been reported in strains D7 (Arni P. et al. ibid. 5, 217-224, 1985), JDI (Brooks T.M. et al. ibid. 5, 225-228, 1985), PV-2 and PV-3 (Inge-Vechtsov S.G. et al. ibid. 5, 243-255, 1985), all treated with and without activation.

The metabolic activation system (S9) was derived from the liver of Aroclor-1254-induced rats. The positive control cells were treated with cyclophosphamide or ethylmethanesulphonate. Dimethylsulphoxide was used for the negative solvent control. The culture was incubated with

benzene or the control chemicals in the absence or presence of S9 for 17 hours. At the end of treatment, the cells were washed, plated and scored.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99% pure.

06-JAN-1997

(764)

**Type:** Yeast Cytogenetic assay

**System of testing:** Saccharomyces cerevisiae strains D6 and D61-M

**Concentration:** see remarks

**Metabolic activation:** with and without

**Result:** positive

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Cells were exposed to benzene either at the time of inoculation into high glucose medium or 4 hours after inoculation. All cultures were then grown for 18 hours on a shaker. The cells were then washed and plated on selective media to detect cell viability and mitotic aneuploidy. Benzene was dissolved in dimethylsulphoxide.

Cyclophosphamide and chenodeoxycholic acid were used as the positive controls. The metabolic activation system (S9) was derived from the liver or Aroclor-induced rats.

Reliability: 2 (valid with restriction)

No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

no data about concentration used

results:

Aneuploidy occurred in D6 cells treated, in the absence of S9, 4 hours after inoculation into high glucose medium and in D61-M cells treated, in the absence and presence of S9, immediately after inoculation into high glucose medium. No effect was seen when this inoculation was delayed 4 hours.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Lancaster synthesis, purity not specified.

06-JAN-1997

(872)

**Type:** Cytogenetic assay

**System of testing:** Chinese hamster fibroblast cell line CH1-L

**Concentration:** 25, 62, 125, 250 ug/ml

**Metabolic activation:** without

**Result:** positive

**Method:** other: in compliance with OECD Guide-line 473

**GLP:** no data

**Test substance:** other TS: purity 99%

**Remark:** method: preliminary cytotoxicity test; triplicate test/concentration; positive and negative solvent control (DMSO); incubation with the test substance for 36 h, colcemid added last 3 h; 2 independent replicates; 300 cells analysed (pos. control 200).  
results: significant hypo- and hyper-diploidy at 62 ug/ml,

highly significant hyper-ploidy at the top concentration.  
Reliability: 1 (valid)  
Comparable to guideline study  
single harvest time

**Source:** Deutsche Shell Chemie GmbH Eschborn (273)  
06-JAN-1997

**Type:** Mammalian cell gene mutation assay  
**System of testing:** human lymphoblast cell lines TK6 and AHH-1  
**Concentration:** 0.5, 1, 1.5, 2 mg/ml  
**Metabolic activation:** without  
**Result:** positive

**Method:** other: in compliance with OECD Guide-line 476  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS: purity 99%

**Remark:** method: preliminary cytotoxicity determination; at least 1 independent replicate; positive and negative solvent control; TK6 cells with metabolic activation (exposure 3 h), AHH-1 cells without (exposure to benzene for 28 h); phenotypic expression period 3 d for thymidine kinase locus in TK6 and 6 d for HGPRT locus in AHH-1 cells. results: significantly positive results at  $\geq 1$  mg/ml in AHH-1 cells.  
Reliability: 1 (valid)  
Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn (255)  
06-JAN-1997

**Type:** other: DNA adducts  
**System of testing:** HL-60 cells and human bone marrow  
**Concentration:** 100  $\mu$ M, 250  $\mu$ M p-benzoquinone, 500  $\mu$ M hydroquinone  
**Metabolic activation:** without  
**Result:** positive

**Method:** other: no guideline available  
**Year:** 1993  
**GLP:** no  
**Test substance:** other TS: p-benzoquinone hydroquinone

**Remark:** Examined DNA adduct formation in HL-60 cells and human bone marrow treated with either hydroquinone or p-benzoquinone and found that these treatments produce the same DNA adduct in both cell types. The DNA adduct level from these treatments varied from 0.05 to 7.5 adducts per 10(7) nucleotides as a function of treatment time and concentration for both compounds. Reaction of calf thymus DNA with p-benzoquinone produced three adducts as detected by  $^{32}$ P-postlabeling. These adducts have been identified as (3'-hydroxy)-3,N(4)-benzetheno-2'-deoxycytidine-3'-phosphate ; (3'-hydroxy)-1,N(6)-benzetheno-2'-deoxyadenosine-3'-phosphate; and (3'-hydroxy)-1,N(2)-benzetheno-2'-deoxyguanosine-3'-phosphate. The DNA adduct formed in HL-60 cells did not correspond

to any of the principal adducts formed in DNA reacted with p-benzoquinone, suggesting that cellular environment modifies DNA adduct production by p-benzoquinone. These studies demonstrate that DNA adduct formation occurs in human bone marrow treated with benzene metabolites.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (122)

**Type:** Cytogenetic assay  
**System of testing:** Human lymphocytes, Chinese hamster lung (CHL) and Chinese hamster ovary (CHO) cells  
**Concentration:** ~10-1100 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** other: nonguideline  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Positive results were obtained only when mitotic delay was taken into account. Tests for sister-chromatid-exchanges were negative.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (285)

**Type:** Sister chromatid exchange assay  
**System of testing:** human T-lymphocytes  
**Concentration:** 5 - 7000 uM  
**Metabolic activation:** without  
**Result:** positive

**Method:** other: nonguideline  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS: as prescribed by 1.1-1.4 and metabolites

**Remark:** Low-dose benzene exposure (10 to 28 ppm for 4 to 6 h) in mice can induce sister chromatid exchange (SCE) in peripheral blood B-lymphocytes. Induced SCE frequencies, mitotic indices, and cell cycle kinetics were quantitated in human peripheral blood T-lymphocytes exposed to benzene, phenol, catechol, 1,2,4-benzenetriol, hydroquinone, 1,4-benzoquinone, or trans,trans-muconic acid. Benzene, phenol, catechol, 1,2,4-benzenetriol, hydroquinone, and 1,4-benzoquinone induced significant concentration-related increases in the SCE frequency, decreases in mitotic indices, and inhibition of cell cycle kinetics. Based on the slope of the linear regression curves for SCE induction, the relative potencies were as follows: catechol > 1,4-benzoquinone > hydroquinone > 1,2,4-benzenetriol > phenol > benzene. On an induced SCE per uM basis, catechol was approximately 221 times more active than benzene at the highest concentrations studied. trans,trans-Muconic acid had no significant effect on the cytogenetic parameters analyzed.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (339)

**Type:** other: DNA adducts  
**System of testing:** In vitro in DNA and cell Culture and in vivo  
**Concentration:** varied  
**Metabolic activation:** without

**Method:** other: nonguideline  
**Year:** 1994  
**GLP:** no data  
**Test substance:** other TS: as prescribed by 1.1-1.4 and metabolites

**Remark:** The metabolites of benzene; hydroquinone, benzoquinone and trans,trans-muconaldehyde produce several adducts when reacted with DNA as detected by the 32P-postlabelling assay. Treatment of rats with benzene resulted in a dose related increase of a single DNA adduct in several organs including the liver. However, in another study in which rats were treated with 200-500 mg/kg for up to 10 weeks, DNA adducts were not consistently detected in several organs. Preliminary results from a recent study suggest that phenol or hydroquinone at a dose level of 50-100 mg/kg can cause DNA adducts in the bone marrow of rats detectable by the 32P-postlabelling method.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(1282)

**Type:** Sister chromatid exchange assay  
**System of testing:** Chinese hamster ovary cells with and without rat liver metabolic activation  
**Concentration:** 750 ug/ml  
**Metabolic activation:** with and without  
**Result:** positive

**Method:** OECD Guide-line 479  
**Year:** 1986  
**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Four widely used in vitro assays for genetic toxicity were evaluated for their ability to predict the carcinogenicity of selected chemicals in rodents. These assays were mutagenesis in Salmonella and mouse lymphoma cells and chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells. Test results from the four in vitro assays did not show significant differences in individual concordance with the rodent carcinogenicity results; the concordance of each assay was approximately 60 percent. There was no evidence of complementarity among the four assays, and no battery of tests constructed from these assays improved substantially on the overall performance of the Salmonella assay. The in vitro assays which represented a range of three cell types and four end points did show substantial agreement among themselves, indicating that chemicals positive in one in vitro assay tended to be positive in the other in vitro assays. However, benzene was positive only in the SCE assay without metabolic activation.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1120)

**Type:** Cytogenetic assay  
**System of testing:** Chinese hamster ovary cells with and without rat liver metabolic activation  
**Metabolic activation:** with and without  
**Result:** negative

**Method:** OECD Guide-line 473  
**Year:** 1983  
**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** The results of a recent comprehensive evaluation of the relationship between four measures of in vitro genetic toxicity and the capacity of the chemicals to induce neoplasia in rodents carry some important implications. The results showed that while the Salmonella mutagenesis assay detected only about half of the carcinogens as mutagens, the other three in vitro assays (mutagenesis in MOLY cells or induction of aberrations or SCEs in CHO cells) did not complement Salmonella since they failed to effectively discriminate between the carcinogens and noncarcinogens found negative in the Salmonella assay. The specificity of the Salmonella assay was relatively high (only 4 of 29 non-carcinogens were positive). Benzene was negative for the induction of chromosome aberrations in vitro. However, benzene was positive in the CHO cell SCE assay without rat liver metabolic activation.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1119)

**Type:** other: Micronucleus in vitro  
**System of testing:** human lymphocytes and human myeloid HL60 cells  
**Concentration:** 0 to 100 uM  
**Metabolic activation:** without  
**Result:** positive

**Method:** other: no guideline  
**GLP:** no data  
**Test substance:** other TS: 1,2,4-benzenetriol (99%)

**Remark:** The triphenolic metabolite of benzene, 1,2,4-benzenetriol (BT), is readily oxidized to its corresponding quinone via a semiquinone radical. During this process, active oxygen species are formed that may damage DNA and other cellular macromolecules. The ability of BT to induce micronuclei (MN) and oxidative DNA damage was investigated in both human lymphocytes and HL60 cells. BT increased the frequency of MN formation twofold in lymphocytes and eightfold in HL60 cells. A linear dose-related increase in MN, was observed in both HL60 cells and lymphocytes. BT also increased the level of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), a marker of active oxygen-induced DNA damage.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1310)

**Type:** other  
**System of testing:** Swiss Webster mice  
**Concentration:** 10, 20, and 40 micromoles  
**Metabolic activation:** without

**Year:** 1998  
**Test substance:** other TS: benzene metabolites (phenol, hydroquinone, catechol, benzoquinone, and trans, trans-muconic acid)

**Remark:** Benzene, a widely used compound, is a known carcinogen and hematopoietic toxicant. Several studies have shown gender and age differences in the responses to benzene-induced hematotoxicity. It is not known if these differences in response are due to age- or gender-associated metabolic differences or to age- or gender-associated differences in the susceptibilities of the target cells. In order to address this issue, mouse colony-forming units-erythroid (CFU-e, an erythroid precursor cell particularly susceptible to benzene toxicity) were cultured in the presence of either individual benzene metabolites or binary mixtures of these metabolites. CFU-e were obtained from unexposed age-matched adult male and female (both virgin and pregnant) Swiss Webster (SW) mice and from SW male and female 16-day fetuses. The metabolites used were phenol, hydroquinone, catechol, benzoquinone, and trans, trans-muconic acid. The concentrations of the individual metabolites used were 10, 20, and 40 microM. Binary mixtures of metabolites were prepared using the lowest concentrations of the individual metabolites that caused cytotoxicity. These concentrations were 10 microM for hydroquinone, catechol, and benzoquinone, and 40 microM for phenol and muconic acid. In general, the CFU-e from adult females (both virgin and pregnant) were more resistant to the toxic effects of the individual metabolites than CFU-e from other subjects. CFU-e from adult males were more susceptible to the cytotoxic effects of hydroquinone and benzoquinone than CFU-e from other subjects and CFU-e from both male and female fetuses were highly sensitive to the toxic effects of catechol. On the other hand, CFU-e from adult males were less susceptible to the cytotoxic effects of catechol than CFU-e from other subjects. Similar results were observed with binary mixtures of metabolites. CFU-e from adult males were more susceptible to the binary mixtures than CFU-e from virgin females and CFU-e from fetal males were more susceptible than CFU-e from fetal females. In addition, CFU-e from fetuses were more 'resistant than CFU-e from adults to the cytotoxic effects of those binary mixtures that did not contain catechol. In contrast, binary mixtures containing catechol were more toxic to fetal cells than to adult cells. These results suggest that differences in benzene hematotoxicity associated with gender and age may be due, at least in part, to intrinsic factors at the level of the target cell rather than solely to age- or gender-related differences in the metabolism of benzene.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

04-NOV-1998

(246)

**Type:** other: In Vitro Cytotoxicity  
**System of testing:** Hela cells  
**Concentration:** 31.25 - 500 micromolar  
**Metabolic activation:** without  
**Result:** positive

**Method:** other  
**Year:** 1998  
**GLP:** yes  
**Test substance:** other TS: BTEX metabolites (3-, and 4-methylcatechol; 4-hydroxybenzoic acid; 4-hydroxy-3-methoxybenzoic acid)

**Remark:** Fuel leakage from underground storage tanks is a major source of groundwater contamination. Although the toxicity of regulated compounds such as benzene, toluene, ethylbenzene, and xylene (BTEX) are well recognized, the cytotoxicity of their metabolites has not been studied extensively. In this study, Hela cells, propagated at 37 degrees C in an atmosphere of 5% CO<sub>2</sub>-95% air, served as a target for evaluation of cytotoxicity of BTEX metabolites 3-methylcatechol, 4-methylcatechol, 4-hydroxybenzoic acid, and 4-hydroxy -3-methoxybenzoic acid. The cells were exposed to different concentrations of the metabolites, which subsequently showed inhibition of cell growth and produced dose-related decreases in cell viability and cell protein content. The BTEX metabolites affected the levels of the polyamines spermidine, spermine, and putrescine, which are known to be important in cell proliferation. The cytotoxic effects for these BTEX metabolites to Hela cells were 3-methylcatechol > 4-methylcatechol > -hydroxy -3-methoxybenzoic acid > 4-hydroxybenzoic acid.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

04-NOV-1998

(1033)

### 5.6 Genetic Toxicity 'in Vivo'

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** up to 48 h  
**Doses:** up to 1 ml/kg  
**Result:** positive

**Method:** OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(210)

**Type:** other: DNA damage in lymphocytes (comet assay)  
**Species:** human **Sex:**

**Method:** SUBJECTS AND METHODS  
359 workers (252 male, 107 female) were recruited from a modern elevator manufacturing facility. 205 were from production departments and 154 from managerial departments. Information on the workers health, smoking habits, alcohol intake and occupational exposure history was collected by personal interview. Blood was collected by venipuncture into heparinised tubes "in the morning", coded and stored at 4 degrees C until analysis (within 6 hr of collection).

#### COMET ASSAY

Lymphocytes were separated from whole blood and DNA damage measured after separation on 4-layer agarose slides with computerised image analysis (- Comet assay: limited information contained in publication). Tail moment (50 measurements per subject) was used as the measure of DNA damage.

#### STATISTICAL ANALYSIS

Tail moments were log-transformed and analysed by independent t-tests, ANOVA and ANCOVA.

**Remark:** Much of the analysis presented in this paper focuses on the relationship between smoking and DNA damage in lymphocytes. Overall, low-level benzene exposure in the workplace makes a relatively minor contribution compared to that seen with smoking.

**Result:** Benzene exposure among the elevator manufacturing workers was in a range 0.5-3.2 mg/m<sup>3</sup>, versus a MAC of 40 mg/m<sup>3</sup>.

Manufacturing workers had larger tail moments than managerial workers (tail moment of 0.53 versus 0.74  $\mu\text{m}$ ).

Tail moments were increased by alcohol consumption, smoking and passive smoking in both groups. Analysis by ANCOVA (using occupational benzene exposure, smoking, passive smoking, alcohol consumption, gender and age as covariates) showed a highly significant difference in tail moment ( $P=0.000$ ) between non-smoking production- (0.78  $\mu\text{m}$ ) and management (0.58) workers, however the largest tail moment was seen in daily smokers (0.93  $\mu\text{m}$ ), followed by occasional smokers (0.77  $\mu\text{m}$ ) and ex-smokers (0.70  $\mu\text{m}$ ).

The manufacturing workers were also exposed to low levels of other aromatic solvents (toluene 1.2-22.3 mg/m<sup>3</sup>; xylene, 0.5-12.0 mg/m<sup>3</sup>), leading the authors note that it is not possible to identify which specific chemical exposure was responsible to the effects noted in the non-smokers.

**Source:** A.K. Mallett Surrey

**Conclusion:** Increased DNA damage was found in lymphocytes from workers with low-level exposure to benzene and other aromatic solvents. It was not possible to identify the cause of this increase. Smoking had a much more pronounced effect.

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male/female  
**Strain:** Long-Evans  
**Route of admin.:** other: i.p. and inhalation  
**Exposure period:** up to 24 h  
**Doses:** up to 500 µl/kg or 60 ppm, respectively  
**Result:** positive

**Method:** OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"  
**Year:** 1992  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (379)

**Type:** other: chromosomal alterations in mice  
**Species:** mouse **Sex:**

**Method:** ANIMALS AND TREATMENTS  
Male B6C3F1 mice (age 9 wk; n = 6/group) were given benzene by oral gavage at doses of 0, 50, 100 or 400 mg/kg bwt/d, 5d/wk for 2 wk. Other groups received 0, 100 or 400 mg/kg bwt/d for 6 wk (n = 4/group) or 12 wk (n = 10/group). Animals were sacrificed 24 hr after final treatment, and blood (cardiac puncture) and bone marrow (femoral) preparations prepared using conventional methods.

CONVENTIONAL MICRONUCLEUS ASSAY (ACRIDINE ORANGE STAINING)  
Bone marrow preparations were stained with acridine orange, randomised and examined using fluorescence microscopy (x1250 magnification). A minimum of 1000 normochromic erythrocytes (NCEs) and 1000 polychromatic erythrocytes (PCEs) were scored per animal. For the PCE:NCE ratio, the number of NCEs per 200 PCEs was determined.

PROBES, PROBE GENERATION AND LABELLING  
DNA probes hybridising to the minor- and major satellite sequences of all mouse chromosomes (with the exception of the alpha-chromosome) were generated using polymerase chain reaction techniques.

MODIFIED MICRONUCLEUS ASSAY WITH MOUSE MINOR AND MAJOR SATELLITE PROBES  
Fixed bone marrow cells were treated with a hybridisation cocktail containing probes specific for minor (digoxigenin-11-dUTP-labelled)- and major (Cy3-dUTP-labelled) satellite sequences present in mouse DNA. A minimum of 2000 erythrocytes were scored for micronuclei, and a multicolour fluorescence in situ hybridisation (FISH) technique (using antibody to digoxigenin or Cy3) used to distinguish between regions binding the probes within the micronuclei.

## FLUORESCENCE IN SITU HYBRIDIZATION WITH CHROMOSOME-SPECIFIC DNA PROBES

Bone marrow preparations were hybridised with probes specific for chromosomes 8 or 14, which were visualised using a FISH technique with fluorescein isothiocyanate-avidin to detect the probe DNA. A minimum of 1000 mononuclear- and 1000 polymorphonuclear bone marrow cells were scored using fluorescence microscopy. Cells with 3 or 4 hybridization regions for the chromosome of interest were classified as hyperploid.

## STATISTICAL ANALYSIS

The micronucleus and hyperploidy data were analysed using liner regression or ANOVA of square root transformed data. Protected Fisher least significant difference was used as a post hoc test to identify significant differences between individual treatments.

**Result:**

## SHORT-TERM (2 WK) BENZENE TREATMENT

There was a strong dose-related increase in the frequency of micronuclei in newly-formed PCEs from benzene-treated mice (4% in control versus 30% in animals given 400 mg/kg bwt over 2wk). A similar but less pronounced increase in micronuclei also occurred in NCEs (3% in controls versus 10% at 400 mg/kg bwt/d).

Multicoloured FISH with the mouse major and minor satellites revealed an increase in micronuclei originating from chromosomal loss (7-fold increase at 400 mg/kg bwt/d) as well as micronuclei arising from breakage of mouse heterochromatin (5-fold increase) and euchromatin (6-fold increase) against an overall 4-fold increase in total micronuclei.

DNA probe assays for chromosomes 8 and 14 showed that the frequency of hyperploidy was variable but remained close to control values in all benzene treatment groups, however small (but significant) increases were occasionally detected. When results for the two probes and the two cell types were combined a significant, weak, dose-related increase in frequency of hyperploidy was apparent (2% in controls, <4% in high dose mice). The percentage of mononuclear cells decreased from 60% to 51% (significant) in mice given 400 mg/kg bwt/d over 2 wk.

## MEDIUM-TERM (6 WK) BENZENE TREATMENT

The frequency of micronuclei in PCE increased significantly from 3% in controls to 63% in mice give 400 mg/kg bwt/d for 6 wk, while the frequency in NCEs increased from 2% to 23%. There was a significant decrease in the PCE:NCE ratio (controls = 1.3; 0.4 in high-dose group).

Probes for the mouse major and minor satellites showed an increase in micronuclei originating from chromosomal loss (9-fold increase at 400 mg/kg bwt/d) as well as micronuclei arising from breakage of mouse heterochromatin (18-fold increase) and euchromatin (11-fold increase) against an

overall 12-fold increase in total micronuclei.

DNA probe assays showed no consistent, significant increase in hyperploidy at chromosomes 8 and 14, although values from treated animals were typically slightly higher than control. Combining results for the two probes and the two cell types again showed a significant, weak, dose-related increase in frequency of hyperploidy (15% in controls, 25% in high dose mice). The percentage of mononuclear cells was slightly decreased in benzene-treated animals, but this was significant only in the 100 mg/kg bwt/d group.

#### LONG-TERM (12 WK) BENZENE TREATMENT

The frequency of micronuclei in newly-formed PCEs from benzene-treated mice increased from 2% in control to 75% in animals given 400 mg/kg bwt over 12wk, while the frequency in NCEs increased from 2% to 26%. There was a significant decrease in the PCE:NCE ratio (controls = 1.2; 0.5 in high-dose group).

Probes for the mouse major and minor satellites showed an increase in micronuclei originating from chromosomal loss (26-fold increase at 400 mg/kg bwt/d) as well as micronuclei arising from breakage of the mouse heterochromatin (17-fold increase) and euchromatin (16-fold increase) against an overall 17-fold increase in total micronuclei. At the highest dose (400 mg/kg bwt/d), chromosomal breakage was responsible for approx. 87% of the total micronuclei.

DNA probe assays for chromosomes 8 and 14 generally failed to detect significant increases in hyperploidy. Combining results for the two probes and the two cell types lead to a significant increase in hyperploidy in bone marrow cells from treated mice (12% in controls, 20% in high dose mice). The percentage of mononuclear cells decreased from 45% in controls to 35-32% (significant) in mice given 100 or 400 mg/kg bwt/d over 12 wk.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

Results from these experiments suggest that benzene causes both chromosomal breakage and aneuploidy in mouse bone marrow cells. Chromosomal breakage is the predominant effect and occurred primarily within euchromatin. Aneuploidy was relatively infrequent, with increases of both chromosome loss and hyperploidy being observed.

26-JAN-2003

(319)

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male  
**Strain:** Wistar  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 100 or 1000 ppm  
**Result:** positive

**Method:** OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"  
**Year:** 1984  
**GLP:** no  
**Test substance:** other TS

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Test substance:** Laboratory reagent grade; purity not specified.  
**Flag:** Risk Assessment  
21-AUG-2000 (1098)

**Type:** other: chromosomal alterations in benzene-exposed humans  
**Species:** human **Sex:**

**Method:** SUBJECTS AND METHODS  
Blood samples were obtained from 17 workers from a shale oil plant and 8 unexposed donors from Estonia. 12 of the shale workers were involved in benzene production, the other 5 in coke oven operations. Blood samples were taken by venipuncture and used to prepare smears which were stored at -20 degrees until use. 48-hr lymphocyte cultures were established from heparinised whole blood, fixed and stored at - 20 degrees (up to 1 yr) for use in in situ hybridisation studies. Data were collected on the concentration of benzene in workplace air and blood, as well as the level of t,t-muconic acid and S-phenylmercapturic acid present in urine (methods not stated).

Additional slides containing fixed, 72-hr cultured lymphocytes were obtained from a Chinese worker population (part of a joint study between the US National Cancer Institute and the Chinese Academy of Preventative Medicine). One set of samples was obtained from 44 workers with current benzene exposure and 44 age-matched controls from the same region of Shanghai, China. A second set of samples was collected from a group of 50 individuals who had previously shown evidence of benzene 'poisoning' (no further details available) and 50 age matched controls.

PROBES AND LABELLING  
A dioxigenin-labelled alpha-satellite probe (to target the centromeric region) and a Cy3-labelled classical satellite probe (to target adjacent pericentric heterochromatin) were used to label chromosome 1. An alpha satellite probe for chromosome 9 was generated by polymerase chain reaction using genomic DNA and oligotide primers.

## FLUORESCENCE IN SITU HYBRIDISATION

Red blood cells collected from the Estonian subjects were removed from the smears (methanol:acetic acid), dehydrated and then hybridised (Rupa et al. (1995) *Cancer Res*, 55, 640). 48-hr cultured lymphocytes from the same study and 72-hr cultured lymphocytes from the Chinese workers were treated with saponine, pepsin and protease K before dehydration of the slide-fixed preparations.

Granulocytes and unstimulated lymphocytes in the blood smears plus the 48-hr cultured lymphocytes were subject to analysis using tandem fluorescence in situ hybridisation (FISH) using alpha-satellite probes and classical-satellite probes for chromosomes 1 or 9. The dioxigenin-labelled alpha-satellite probes were localised using mouse anti-dioxigenin immunoglobulin G, followed by amplification with dioxigenin-conjugated sheep anti-mouse antibody and treatment with FITC-conjugated sheep anti-dioxigenin IgG. Slides were scored blind using fluorescence microscopy, and the frequencies of alterations determined by counting 1000 cells per individual for lymphocytes and 500 cells per cell type for the blood smear mononuclear- and polymorphonuclear cells.

## STATISTICAL ANALYSIS

Chromosome breakage data were subject to square root transformation and analysed by simple and multiple regression, ANOVA, Protected Fisher least significant difference test and Student's t test. Hyperploidy results from the Estonian population were compared using Kruskal-Wallis ANOVA, Spearman rank correlation and Mann-Whitney U test due to a large number of zero values.

**Result:**

CHROMOSOMAL ALTERATIONS IN BENZENE-EXPOSED ESTONIAN WORKERS  
Benzene exposure in Estonian benzene production workers (4.1 mg/m<sup>3</sup>) and coke over workers (1.1 mg/m<sup>3</sup>) was greater than that of controls (no detectable exposure). Blood benzene levels of 86, 54 and 22 nmol/l, respectively, were recorded for these groups. Urinary t,t-muconic acid and S-phenylmercapturic acid followed blood benzene levels (22, 5 or 1 umol/l for t,t-muconic acid and 40, 13 or 3 umol/l for S-phenylmercapturic acid).

Blood analyses revealed that breakages in the labelled region of chromosome 1 were generally higher in granulocytes than in unstimulated lymphocytes, however statistical analysis showed there was no significant difference between the two benzene-exposed groups and the controls. There was also no significant association between the incidence of chromosomal breakage (either in lymphocytes or granulocytes) and age, smoking habits, markers of benzene exposure or length of employment.

Tandem labelling analysis of chromosome 1 in cultured lymphocytes (48-hr culture period) showed a modest but significant increase in breakage in the 1cen-1q12 region in cells from benzene production workers (median frequency = 6%) compared with both the non-exposed controls (2%) and

coke oven workers (4%) exposed to lower levels of benzene. The median incidence of breaks affecting the 9cen-9q12 region of chromosome 9 was significantly greater for benzene production workers (10%) versus the controls (6%), but not for coke oven workers (7%).

There was no correlation between breakage in either the 1q12 or 9q12 regions and biomarkers of exposure, age or smoking status. However an analysis of individual results showed a strong linear correlation between results obtained for the two chromosomes suggesting the method and results were reliable and reproducible.

There was no association between exposure biomarkers and hyperploidy in stimulated lymphocytes from the three groups of workers.

CHROMOSOMAL ALTERATIONS IN BENZENE-EXPOSED CHINESE WORKERS  
Median benzene exposure in Chinese workers was 98 mg/m<sup>3</sup> (8-hr TWA) versus 0.06 mg/m<sup>3</sup> in the control group. Urinary phenol (91 versus 17 ug/g creatinine) and t,t-muconic acid (26 versus 0.2 ug/g creatinine) was higher in the exposed workers than in the controls.

Tandem labelling analysis of chromosome 1 in cultured lymphocytes (72-hr culture period) showed no difference in the frequency of breakage at the 1cen-q12 region of the control (median frequency = 2%) and exposed (2%) populations. The median frequency of hyperploidy was also 2% in both groups. Further statistical analysis of data from the benzene-exposed group suggested a possible weak association between hyperploidy and benzene exposures in excess of the 38 mg.m<sup>3</sup> median (median frequency = 3% in this sub-group of 21 workers versus 2% in controls; statistical significance not given). Linear regression revealed separate, significant associations between urinary phenol or t,t-muconic acid and hyperploidy of chromosome 1 in both the exposed workers and the entire study population. There was no association between urinary biomarkers and chromosome breakage.

CHROMOSOMAL ALTERATIONS IN CHINESE WORKERS PREVIOUSLY 'POISONED' WITH BENZENE

No significant increase in hyperploidy or breakage was seen when cultured lymphocytes from 'poisoned' workers were compared with controls (median frequency of breakage in the 1cen-q12 region = 2% in both groups; median frequency of hyperploidy = 2% in controls and 3% in 'poisoned' individuals).

**Source:** A.K. Mallett Surrey

**Conclusion:** Chromosomal breakage data from these worker population studies are somewhat inconsistent, with an increased frequency recorded in Estonian workers exposed to relatively modest levels of benzene while a more heavily-exposed group of Chinese workers showed no measurable effect. In contrast,

an association between lymphocyte hyperploidy and urinary excretion of benzene metabolites was present in Chinese workers whereas no association was present in the Estonian group. A subset of Chinese workers also showed an association between increased hyperploidy and airborne benzene exposures > 38 mg/m<sup>3</sup> benzene (8-hr TWA).

04-MAR-2003

(319)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 6 weeks  
**Doses:** 0.1 or 1.0 ppm  
**Result:** ambiguous  
**Method:** other  
**Year:** 1988  
**GLP:** no  
**Test substance:** no data

**Remark:** chromosomal aberration test with lung macrophages  
**Source:** German rapporteur  
**Flag:** Risk Assessment

21-AUG-2000

(57)

**Type:** other: role of topoisomerase II in benzene-related DNA damage

**Method:** IN VITRO TOPOISOMERASE INHIBITION ASSAYS  
 Inhibition of topoisomerase II was followed using gel electrophoresis to visualise monomers released from isolated mitochondrial DNA after incubation with benzene metabolites (commercial kit: TopoGEN). Experiments were performed in duplicate, in the presence and in the absence of a horse radish peroxidase system (HRPS; used to activate metabolites).

BINDING STUDIES USING ISOLATED TOPOISOMERASE II  
 [14C]-Phenol was converted to reactive metabolites using a horse radish peroxidase system and mixed with topoisomerase II, in the absence or presence of additional glutathione. Topoisomerase proteins containing bound radioactivity were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and accelerator mass spectrometry (AMS) used to characterise bound [14C]phenol equivalents.

STUDIES USING HUMAN HL-60 CELLS  
 HL-60 cells (from a human promyelocytic leukemic cell line) were incubated with various concentrations of benzene metabolites and cell viability followed for up to 48 hr. The results were used to estimate doses at which topoisomerase II inhibition would be likely to occur. In a subsequent series of experiments, HL-60 cells were incubated with biphenol (500 uM), hydroquinone (50 uM), catechol (500 uM) or benzenetriol (100 uM) and HRPS, and topoisomerase II extracted and its activity assayed using a commercial kit.

## INHIBITION OF TOPOISOMERASE IN MOUSE BONE MARROW

Male B6C3F1 mice (6 wk) were given 440 mg benzene/kg bwt/d by gavage in corn oil for 3 consecutive days. Mice were killed 24 hr after the final injections and femoral bone marrow collected. Topoisomerase II was extracted from nuclear proteins and its activity assayed.

Binding of [14]C-benzene was investigated in mice given 440 mg/kg bwt/d containing 1.6 mCi/mmol benzene. Nuclear proteins were extracted from bone marrow cells, immuno-precipitated and bound benzene metabolites separated by SDS-PAGE and characterised by AMS.

## STATISTICAL ANALYSIS

Differences in binding activity of [14C]phenol equivalents were determined using ANOVA. Protected Fisher least significant difference was used as a post hoc test to identify significant differences between individual treatments. Differences in topoisomerase II activity were assessed using a one-group t-test. Cell viability data were analysed using a Student t-test.

**Result:**

Extensive data are presented in the published report of this work. A short summary of key findings is given here.

## EFFECT OF BENZENE METABOLITES ON TOPOISOMERASE I/II

1,4 Benzoquinone directly inhibited topoisomerase II activity at concentrations of 10 uM and above, while t,t-muconaldehyde was inhibitory at 100 uM and above. Phenol, 4,4'-biphenol, 2,2'-biphenol, hydroquinone, catechol and 1,2,4'benzene triol inhibited topoisomerase II activity at 10 uM and above in the presence of HRPS but not in its absence. Titration assays (variation in amount of DNA present in assay) demonstrated that inhibition of topoisomerase II by 100 uM phenol or 2,2'-biphenol in the presence of HRPS was partially reversed when the amount of DNA added to the system was increased, suggesting that inhibition by these substances occurred as a result of an interaction between the metabolite and DNA. Other titration studies (variation in amount of topoisomerase II present) revealed that inhibition by bioactivated 4,4'-biphenol was due to a direct effect on the enzyme. Diphenoquinone (a metabolite of biphenol) was found to directly and completely inhibit topoisomerase II at concentrations of 1 uM and above.

## TOPOISOMERASE II BINDING STUDIES

AMS results for protein adduction using [14C] and HRPS showed that approx. 1.2 pmol of [14C]phenol equivalents (that is, oxidative metabolites derived from phenol) were bound per 1 pmol topoisomerase II (versus 0.9 fmol per pmol in the absence of HRPS). The addition of glutathione decreased binding by approx. one third.

## STUDIES USING HUMAN HL-60 CELLS

Based on results from preliminary cell viability (concentration/time) studies, the effect of defined concentrations of benzene metabolites on topoisomerase II

activity was investigated in HL-60 cells. 4,4'-biphenol (500 uM + HRPS, 8 hr) or hydroquinone (50uM + HRPS, 2 hr) decreased enzyme activity by around 50% compared to that of controls. Benzenetriol (100 uM, 2 hr) decreased topoisomerase II activity to the same extent in the absence of HRPS. Catechol (500 uM + HP, 2 hr incubation) was without effect.

#### INHIBITION OF TOPOISOMERASE II IN MOUSE BONE MARROW

The topoisomerase II activity in bone marrow cells from benzene exposed mice (440 mg/kg bwt on 3 consecutive days) was significantly decreased by approx. 40% relative to that of the controls. Analysis of nuclear proteins showed no increase in binding of radiolabelled benzene (or its metabolites) to the 170-kDa topoisomerase II monomer. The authors considered that low recovery of enzyme from mouse femur may have hindered detection.

**Source:** A.K. Mallett Surrey  
**Conclusion:** Benzene metabolites were shown to inhibit topoisomerase II activity in an isolated enzyme system, in a human bone marrow-derived leukemic cell line and in vivo in bone marrow from treated mice.

04-MAR-2003

(319)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 10 to 1000 ppm  
**Result:** positive  
**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1986  
**GLP:** no  
**Test substance:** no data

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment

21-AUG-2000

(338)

**Type:** Micronucleus assay  
**Species:** rat **Sex:** male  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0.1 to 30 ppm  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1986  
**GLP:** no  
**Test substance:** no data

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (338)

**Type:** Sister chromatid exchange assay  
**Species:** mouse **Sex:** male  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 10 to 1000 ppm  
**Result:** positive

**Method:** other  
**Year:** 1986  
**GLP:** no  
**Test substance:** no data

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (338)

**Type:** Sister chromatid exchange assay  
**Species:** rat **Sex:** male  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0.1 to 30 ppm  
**Result:** positive

**Method:** other  
**Year:** 1986  
**GLP:** no  
**Test substance:** no data

**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (338)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** no data  
**Strain:** no data  
**Route of admin.:** gavage  
**Exposure period:** up to 14 days  
**Doses:** 26.6 to 146.6 mg/kg  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1990  
**GLP:** no  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (58)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** no data  
**Route of admin.:** inhalation  
**Exposure period:** 4 to 24 months  
**Doses:** 25 to 600 mg/kg  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1990  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
14-AUG-2000 (717)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** 24 h  
**Doses:** up to 440 mg/kg  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1994  
**GLP:** no data  
**Test substance:** other TS

**Source:** German Rapporteur  
**Test substance:** purity >99%  
**Flag:** Risk Assessment  
14-AUG-2000 (187)

**Type:** other: alkaline comet assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** gavage  
**Exposure period:** 6 h  
**Doses:** up to 450 mg/kg  
**Result:** positive

**Method:** other: non-routine test system  
**Year:** 1996  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
16-AUG-2000 (1168)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 6 weeks  
**Doses:** 0.04-1.00 ppm  
**Result:** ambiguous

**Method:** OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (59)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** B6C3F1  
**Route of admin.:** inhalation  
**Exposure period:** up to 8 weeks  
**Doses:** up to 200 ppm  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1996  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
15-AUG-2000 (345)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** other: cyp2e1-knockout mice , wild-type and B6C3F1  
**Route of admin.:** inhalation  
**Exposure period:** 5 days  
**Doses:** 200 ppm  
**Result:** positive

**Method:** OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"  
**Year:** 1996  
**GLP:** no data  
**Test substance:** other TS

**Source:** German rapporteur  
**Test substance:** purity 99%  
**Flag:** Risk Assessment  
16-AUG-2000 (1197)

**Type:** Somatic mutation assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 22 hours per day/7 days per week/6 weeks  
**Doses:** 0.04-1000 ppb  
**Result:** ambiguous

**Method:** other  
**Year:** 1992  
**GLP:** no data  
**Test substance:** no data

**Method:** gene mutation test (HPRT variants) in spleen lymphocytes  
**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (1236)

**Type:** other: transgenic mouse gene mutation assay  
**Species:** mouse **Sex:** male  
**Strain:** other: C57BL/6  
**Route of admin.:** inhalation  
**Exposure period:** 12 weeks  
**Doses:** 300 ppm  
**Result:** positive

**Method:** other: non-routine test system  
**Year:** 1995  
**GLP:** no data  
**Test substance:** other TS

**Source:** German rapporteur  
**Test substance:** benzene analytical grade  
**Flag:** Risk Assessment  
21-AUG-2000 (803)

**Type:** Mammalian germ cell cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** up to 48 h  
**Doses:** up to 1 ml/kg  
**Result:** positive

**Method:** OECD Guide-line 483 "Genetic Toxicology: Mammalian Germ-cell Cytogenetic Assay"  
**Year:** 1991  
**GLP:** no data  
**Test substance:** no data

**Remark:** chromosomal aberration assay (spermatogonia)  
**Source:** German rapporteur  
**Flag:** Risk Assessment  
16-AUG-2000 (207)

**Type:** other: transplacental micronucleus test  
**Species:** mouse **Sex:** female  
**Strain:** ICR  
**Route of admin.:** i.p.  
**Exposure period:** 24 h  
**Doses:** 880 to 1760 mg/kg  
**Result:** positive

**Method:** other: non-routine test system  
**Year:** 1989  
**GLP:** no  
**Test substance:** other TS

**Remark:** analysis of polychromatic erythrocytes in fetal livers  
**Source:** German rapporteur  
**Test substance:** from Fisher  
**Flag:** Risk Assessment  
16-AUG-2000 (482)

**Type:** other: transplacental micronucleus test  
**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of admin.:** i.p.  
**Exposure period:** 21 h  
**Doses:** 109 to 874 mg/kg  
**Result:** positive

**Method:** other: non-routine test system  
**Year:** 1991  
**GLP:** no  
**Test substance:** other TS

**Method:** analysis of polychromatic erythrocytes in fetal livers  
**Source:** German rapporteur  
**Test substance:** purity >99,9%  
**Flag:** Risk Assessment  
21-AUG-2000 (833)

**Type:** other: transplacental micronucleus test  
**Species:** mouse **Sex:** female  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** 40 h  
**Doses:** 439 to 1318 mg/kg  
**Result:** positive

**Method:** other: non-routine test system  
**Year:** 1992  
**GLP:** no  
**Test substance:** other TS

**Remark:** analysis of polychromatic erythrocytes in fetal livers  
**Source:** German rapporteur  
**Test substance:** from Baker  
**Flag:** Risk Assessment  
16-AUG-2000 (1287)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** no data  
**Route of admin.:** oral unspecified  
**Exposure period:** 2 weeks  
**Doses:** 36.6- 146.4 mg/kg bw/day  
**Result:** positive

**Method:** other  
**Year:** 1987  
**GLP:** no data  
**Test substance:** no data

**Remark:** in vivo chromosomal aberration assay on spermatocytes  
**Source:** BP Chemicals Ltd LONDON  
German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (945)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** no data  
**Route of admin.:** gavage  
**Exposure period:** 5 weeks  
**Doses:** other: not specified  
**Result:** negative

**Method:** other  
**Year:** 1985  
**GLP:** no data  
**Test substance:** no data

**Method:** no detailed description of methodology and results  
**Source:** German rapporteur  
**Flag:** Risk Assessment  
21-AUG-2000 (351)

**Type:** Dominant lethal assay  
**Species:** rat **Sex:** male  
**Strain:** no data  
**Route of admin.:** i.p.  
**Exposure period:** not given  
**Doses:** 0.5 ml/kg bodyweight  
**Result:** negative

**Method:** other  
**Year:** 1975  
**GLP:** no data  
**Test substance:** no data

**Source:** German rapporteur  
**Flag:** Risk Assessment  
 16-AUG-2000 (715)

**Type:** other  
**Species:** mouse **Sex:** male  
**Strain:** other  
**Route of admin.:** i.p.  
**Exposure period:** 5 days  
**Doses:** 0.4- 1.0 ml/kg bw/day  
**Result:** positive

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Method:** sperm abnormality test with (CBA x BALB/c) F1 mice  
**Source:** BP Chemicals Ltd LONDON  
 German rapporteur  
**Test substance:** Laboratory reagent grade; purity not specified.  
**Flag:** Risk Assessment  
 21-AUG-2000 (1146)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 10.2, 25.6, 64, 160, 400, 2500 µl/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** 813: reason why not..  
 Benzene was administered in peanut oil. Groups of ten male and ten female Swiss Lane Petter mice were exposed to each benzene dose. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were

**Result:** removed and processed for metaphase analysis. The frequency of chromosome aberrations was increased in the benzene treated animals, this being significant at 56.3 mg/kg bw/day and above. The male animals were more sensitive than the females. At doses up to 56.3 mg/kg bw/day, the abnormalities consisted of chromatid gaps and single breaks. At 140 mg/kg bw/day, multiple breaks, as well as gaps and single breaks, occurred and at the higher doses, cells showing pulverisation of chromosomes were also seen. Chromosomal damage has also been reported in Swiss CD-1 mice administered benzene by gavage on two or three days by Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984 and Meyne J. & Legator M.S. Envir. Mutagen 2, 43-50, 1980.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** "chemically pure" benzene tested.

10-AUG-2000

(1042)

**Type:** Cytogenetic assay

**Species:** hamster

**Sex:** male/female

**Strain:** other

**Route of admin.:** gavage

**Exposure period:** 24 hours

**Doses:** 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.

**Result:** In the males, a dose of 8.8 g/kg bw/day induced a significant increase in the frequency of chromosome aberrations, including gaps. In the low-dose group, the frequency of aberrations did not differ significantly from the controls. No significant effect was seen in the females at either dose level.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** "Chemically pure" benzene tested.

13-DEC-1996

(1042)

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 13 weeks  
**Doses:** 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Report of a study conducted for the American Petroleum Institute.

**Result:** No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or less. Slight increases in aberrations were seen at 0.98 mg/l but these were not statistically significant.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (321) (490)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 4 hours  
**Doses:** 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six animals/sex. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrow flushed out, slides prepared and chromosomal aberration frequency assessed.

**Result:** Benzene alone did not increase the frequency of chromosomal aberrations. Treatment with phenobarbital before benzene exposure caused a statistically significant increase in chromatid-type aberrations. This was not seen in control mice pretreated with phenobarbital.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (1138)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 13 weeks  
**Doses:** 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l0  
**Method:** other  
**GLP:** no data  
**Test substance:** no data  
**Remark:** Report of a study conducted for the American Petroleum Institute.  
**Result:** No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or below. Slight increases in aberrations were seen at 0.98 mg/l but these were only statistically significant in the females.  
**Source:** BP Chemicals Ltd LONDON (321) (491)  
13-DEC-1996

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male  
**Strain:** Wistar  
**Route of admin.:** i.p.  
**Exposure period:** single dose  
**Doses:** 878 mg/kg bw  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS  
**Remark:** Groups of five male Wistar-derived, outbred, Alderely Park rats were injected with benzene or, in the case of the control group, water. The animals were killed 24 hours later, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267-1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.  
**Result:** Benzene caused a statistically significant increase in the numbers of cells with abnormalities and chromatid and chromosome gaps and breaks.  
**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; purity not specified. (31)  
13-DEC-1996

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** 3 days  
**Doses:** 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was diluted in corn oil to the required concentration and administered to five males and five females/dose. The control group received corn oil. The mice were treated 2 hours after the final treatment with colchicine and killed 2 hours later. The femurs were removed and the bone marrow prepared and assessed.

**Result:** An increased incidence of chromosome aberrations were seen in the benzene treated mice, the majority of the aberrations being chromatid breaks. The males were more susceptible at all doses than the females. Not all of the increased incidences were found to be statistically significant. Gad-El-Karim and co-workers also reported an increase in chromosome damage in this strain of mouse injected with 2 doses of 440 mg/kg bw (Gad-El-Karim M.M. et al. Mutation Res. 135, 225 243, 1984).

**Source:** BP Chemicals Ltd LONDON

**Test substance:** >99% pure.  
13-DEC-1996

(772)

**Type:** Cytogenetic assay  
**Species:** Chinese hamster **Sex:** male/female  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.

Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, one sampling time, 2 doses  
**Result:** In the males, a dose of 8.8 g/kg bw/day induced a significant increase in the frequency of chromosome aberrations, including gaps. In the low-dose group, the frequency of aberrations did not differ significantly from the controls. No significant effect was seen in the females

at either dose level.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** "Chemically pure" benzene tested.  
06-JAN-1997 (1042)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** ICR  
**Route of admin.:** gavage  
**Exposure period:** 2 weeks  
**Doses:** 0, 36.6, 73.2, 146.4 mg/kg bw/day

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of five mice were administered benzene in olive oil daily for 2 weeks excluding days 5 and 10. The mice were killed on day 15 and spleen lymphocytes were isolated and cultured. Slides were prepared and analysed for the presence of chromosome aberrations.  
Reliability: 4 (validity cannot be judged)  
Original reference not yet available

**Result:** Significant dose-dependent increases in both chromatid breaks and abnormal cells were found in the top two dose groups. An increased frequency of breaks and abnormal cells was also recorded at 36.6 mg/kg bw/day but this was not significantly different from the control group. The frequency of cells with multiple breaks was increased at 73.2 and 146.4 mg/kg bw/day and there was a significant increased frequency of polyploid cells at all dose levels.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (944)

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 13 weeks  
**Doses:** 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 4 (validity cannot be judged)  
Original reference not yet available  
Report of a study conducted for the American Petroleum Institute.

**Result:** No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or less. Slight increases in aberrations were seen at 0.98 mg/l but these were not statistically significant.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (321) (490)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: DBA/2  
**Route of admin.:** inhalation  
**Exposure period:** 4 hours  
**Doses:** 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six animals/sex. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrow flushed out, slides prepared and chromosomal aberration frequency assessed.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, one sampling time, no data about the test substance

**Result:** Benzene alone did not increase the frequency of chromosomal aberrations. Treatment with phenobarbital before benzene exposure caused a statistically significant increase in chromatid-type aberrations. This was not seen in control mice pretreated with phenobarbital.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(1138)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: Swiss CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 22 hours per day/7 days per week/6 weeks  
**Doses:** 0, 40, 100, 1000 ppb (converts to 0, 0.13, 0.33, 3.26 ug/l)  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of 14 male and 14 female mice/dose were used. After benzene treatment, the animals were exposed to vinblastine sulphate for 4 hours before sacrifice. A further group of 12 mice/sex were exposed to 0, 0.33 and 3.26 ug/l and injected, 4 hours before sacrifice, with colchicine. After the mice were killed, lymphocytes were obtained from the spleen for cytogenetic analysis. Analytical concentrations: 0, 34, 88, 858 ppb (deviation < 5%). 2 independent experiments. At least 6 mice/dose/sex were analysed for chromosomal aberration.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions

**Result:** no positive control, no data about test substance  
No overt signs of toxicity were seen during the study. Dose-dependent increases in chromosome aberration frequencies (chromatid breaks) were observed in both sexes at the two top doses, this being statistically significant. In the females, the increase after exposure to 3.26 mg/l was much moderated compared to the increase in the 0.33 mg/l dose group. On repeating the experiment and including exposure to 0.13 mg/l, statistically significant increases in the frequencies of aberrations were again seen. However, in both sexes, those in the top-dosed groups were lower than the frequencies seen in mice exposed to the lower benzene concentrations. Other positive results in mice inhaling benzene have been reported by Au W.W. et al. *Envir. Mutagen.* 9(Suppl.8), 8, 1987 (Abstract 16); Tice R.R. et al. *Envir. Sci. Res.* 25, 257-275, 1982; Zhurkov V.S. et al. *Bull. exp. Biol. Med.* 96, 1741-1743, 1983.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (59)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 13 weeks  
**Doses:** 0, 1, 10, 30, 300 ppm (converts to 0, 0.003, 0.033, 0.098, 0.98 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 4 (validity cannot be judged)  
Original reference not yet available  
Report of a study conducted for the American Petroleum Institute.

**Result:** No increase in chromosomal damage was observed at concentrations of 0.098 mg/l or below. Slight increases in aberrations were seen at 0.98 mg/l but these were only statistically significant in the females.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (321) (491)

**Type:** Cytogenetic assay  
**Species:** rat  
**Strain:** Wistar  
**Route of admin.:** i.p.  
**Exposure period:** single dose  
**Doses:** 878 mg/kg bw

**Sex:** male

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of five male Wistar-derived, outbred, Alderely Park rats were injected with benzene or, in the case of the control group, water. The animals were killed 24 hours later, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267-1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.  
Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions no positive control, one sampling time, one sex, one dose  
**Result:** Benzene caused a statistically significant increase in the numbers of cells with abnormalities and chromatid and chromosome gaps and breaks.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; purity not specified.  
06-JAN-1997

(31)

**Type:** Cytogenetic assay  
**Species:** rat  
**Strain:** Wistar  
**Route of admin.:** inhalation  
**Exposure period:** a) single exposure for 2 h , b) 6 h per day for 5 days  
**Doses:** 0, 750 or 7500 ppm (2.4 or 24 mg/l)

**Sex:** male

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of at least 7 male Wistar-derived, outbred, Alderely Park rats were exposed to benzene or, in the case of the control group, to air. The animals were killed 6 (b) or 24 (a and b) hours after last exposure, the bone marrow samples prepared according to the method of Sugiyama T. J. natn. Cancer Inst. 47, 1267-1275, 1971 but with slight modifications and the slides examined for any chromosome abnormalities.  
Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions no positive control, one sex  
**Result:** Benzene caused a statistically significant clastogenic effect in all treatment groups.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; purity not specified.  
06-JAN-1997

(31)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** other: Albino  
**Route of admin.:** inhalation  
**Exposure period:** Continuous exposure for 7 days  
**Doses:** 9.7, 13.9, 36.8, 73.7 mg/m<sup>3</sup>

**Method:** other: Zhurkov et al.  
**Year:** 1983  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Colchicine was administered by i.p. 2 hours prior to sacrifice. One hundred metaphases were evaluated per animal. Benzene concentration had no effect on mitotic activity in the bone marrow. However, the frequency of cells with chromosomal aberrations increased with increasing benzene concentration. The minimal active concentration of benzene was 36.8 mg/m<sup>3</sup>. The maximum inactive concentration was 13.9 mg/m<sup>3</sup>. The principal chromosomal aberrations were single and paired fragments. Single chromatid exchanges were observed. However, there were no chromosomal exchanges. The calculated allowable concentration of benzene that would cause the spontaneous mutation rate to increase by 1% compared to controls equalled 0.06 mg/m<sup>3</sup>. In accordance with the formula MAD/200 proposed by Zhurkov, the allowable concentration of benzene by inhalation was 0.18 mg/m<sup>3</sup>. [MAD = the minimal allowable dose of mutagen causing a significant increase in the mutation level in the experimental group compared to control.]

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(1312)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Strain:** other: Albino  
**Route of admin.:** gavage  
**Exposure period:** 10 consecutive days  
**Doses:** 5, 20 and 80 mg/kg in vegetable oil

**Method:** other: Zhurkov et al.  
**Year:** 1983  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Colchicine was administered by i.p. 2 hours prior to sacrifice. One hundred metaphases were evaluated per animal. Benzene concentration had no effect on mitotic activity in bone marrow. However, the frequency of cells with chromosomal aberrations increased with an increase in benzene concentration. The minimal active dose was 20 mg/kg, and the maximal inactive dose was 5 mg/kg. The principal chromosomal aberrations were single and paired fragments. Single chromatid exchanges were observed. There were no chromosomal exchanges. The calculated allowable concentration of benzene that would cause the

spontaneous mutation rate to increase by 1% compared to controls equalled 0.07 mg/kg. In accordance with the formula MAD/200 proposed by Zhurkov, the allowable concentration of benzene by oral route was 0.1 mg/kg. [MAD = the minimal allowable dose of mutagen causing a significant increase in the mutation level in the experimental group compared to control.]

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1312)

**Type:** Cytogenetic assay  
**Species:** human **Sex:** male/female  
**Route of admin.:** inhalation  
**Exposure period:** 10-23 years  
**Doses:** 0, < 31 ppm

**Method:** other: nonguideline  
**Year:** 1989  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Cytogenetic analysis was carried out on peripheral blood lymphocytes of 33 workers professionally exposed to benzene for 10-23 years. In the examined group structural chromosome aberrations were found in 147 metaphases (4.7%). Statistical analysis has shown that the distribution of breakpoints was not random. Chromosomes 2, 4 and 9 are almost twice as susceptible to breaks, while chromosomes 1 and 2 are almost twice as susceptible to gaps, as would be expected based on a random distribution of damage among chromosomes.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1002)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male  
**Route of admin.:** i.p.  
**Exposure period:** 3 days

**Method:** other: nonguideline  
**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Benzene induced a statistically significant increase of both chromosome aberrations and micronuclei in bone marrow cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1030)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 4 hour  
**Doses:** 3000 ppm

**Method:** other: nonguideline  
**Year:** 1982  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** The different responses to benzene observed in male and in female DBA/2 mice (with and without phenobarbital pretreatment), suggest that different metabolites of benzene are responsible for the induced chromosomal aberrations and SCEs and for the inhibition of cellular proliferation in bone marrow tissue. The results from the partial hepatectomy and toluene studies indicate that while metabolism of benzene is necessary before genotoxic damage can occur in bone marrow cells, the liver may not be the main site of production for the SCE-inducing metabolite. A four-hour exposure to ambient concentrations of benzene as low as 28 ppm induces a significant increase in bone marrow genotoxic damage as measured by SCE formation. The modification of the magnitude of the benzene-induced SCE frequency in murine bone marrow cells by age, sex and genetic constitution emphasizes the importance of considering these parameters when attempting to extrapolate animal data to human health risks.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(1140)

**Type:** Cytogenetic assay  
**Species:** human **Sex:** male/female  
**Route of admin.:** inhalation  
**Exposure period:** 0 to 10 years  
**Doses:** 0 - 22 ppm

**Method:** other: nonguideline  
**Year:** 1994  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** The genotoxic effects of benzene were assessed in peripheral blood lymphocytes of 49 workers occupationally exposed to benzene (0.9-22 ppm in the work environment) for 0-2, 2-10 and more than 10 years. Chromosomal aberrations, SCEs and UV-induced DNA synthesis were used as indicators of genotoxic effects. Most of the workers were followed up in 1991 and 1992, while the benzene concentrations were reduced to 0.3-5.8 ppm. Considered overall, in the "exposed" groups, the frequencies of chromosomal aberrations were significantly higher than in controls thus providing evidence for the clastogenic effects of benzene. However, there seems to be no correlation between aberration frequencies and the duration of prior exposure to benzene.

In 1991 and 1992, when the benzene concentrations were brought down, there was a concomitant decrease in the frequencies of chromosomal aberrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn (1145)  
06-JAN-1997

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: Swiss Lane Petter  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 10.2, 25.6, 64, 160, 400, 2500 ul/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)

**Method:** other: in compliance with OECD Guide-line 475  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was administered in peanut oil. Groups of ten male and ten female Swiss Lane Petter mice were exposed to each benzene dose. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. Animals received an intraperitoneal injection of colchicine 3 hours after the second dose and were killed 3 hours later. The femurs were removed and processed for metaphase analysis.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, one sampling time

**Result:** The frequency of chromosome aberrations was increased in the benzene treated animals, this being significant at 56.3 mg/kg bw/day and above in males and females. The male animals were more sensitive than the females. At doses up to 56.3 mg/kg bw/day, the abnormalities consisted of chromatid gaps and single breaks. At 140 mg/kg bw/day, multiple breaks, as well as gaps and single breaks, occurred and at the higher doses, cells showing pulverisation of chromosomes were also seen. Chromosomal damage has also been reported in Swiss CD-1 mice administered benzene by gavage on two or three days by Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984 and Meyne J. & Legator M.S. Envir. Mutagen 2, 43-50, 1980.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** "chemically pure" benzene tested. (1042)  
06-JAN-1997

**Type:** Cytogenetic assay  
**Species:** rat **Sex:** male  
**Strain:** Wistar  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0, 1, 10, 100, 1000 ppm (converts to 0, 0.003, 0.033, 0.33, 3.26 mg/l)

**Method:** other: in compliance with OECD Guide-line 475  
**Year:** 1984  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Only male animals were tested (justified by the authors). Groups of eight rats were exposed at each benzene concentration and the control group contained 12 animals. Controls were exposed to clean dry air. The benzene exposure concentrations were measured. Animals received colchicine intra-peritoneally 2 hours before sacrifice, they were then killed, their femurs removed and the bone marrow harvested. Slides were prepared and examined for abnormalities.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, single sampling time

**Result:** Analysis of the test atmospheres gave benzene concentrations of 0.0026 ñ 0.0006, 0.032 ñ 0.006, 0.44 ñ 0.12 and 3.56 ñ 0.34 mg/l. The breathing of animals at the top dose was very rapid immediately after the exposure period. This was not reported at the lower doses. There was a statistically significant increase in the percentage of cells with abnormalities, including or excluding gaps, in animals exposed to 0.33 and 3.26 mg/l. Elevated levels of abnormal cells were seen at 0.003 and 0.033 mg/l, these being dose-related but not statistically significant. Positive results in rats inhaling benzene have been reported by other investigators (Anderson D. & Richardson C.R. Mutation Res. 90, 261-272, 1981).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** analytical reagent grade; purity not specified.  
06-JAN-1997 (1098)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: Swiss CD-1  
**Route of admin.:** gavage  
**Exposure period:** 3 days  
**Doses:** 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)

**Method:** other: nearly in compliance with OECD Guide-line 475  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was diluted in corn oil to the required concentration and administered to five males and five females/dose. The control group received corn oil. The mice were treated 2 hours after the final treatment with colchicine and killed 2 hours later. The femurs were removed and the bone marrow prepared and assessed. Reliability: 2 (valid with restriction) Comparable to guideline study with acceptable restrictions no positive control, one sampling time

**Result:** A significantly increased incidence of chromosome aberrations were seen in the benzene high-dosed females and in the mid- and high-dosed male mice, the majority of the aberrations being chromatid breaks. The males were more susceptible at all doses than the females. Gad-El-Karim and co-workers also reported an increase in chromosome damage in this strain of mouse injected with 2 doses of 440 mg/kg bw (Gad-El-Karim M.M. et al. Mutation Res. 135, 225 243, 1984).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** >99% pure.  
 06-JAN-1997 (772)

**Type:** Cytogenetic assay  
**Species:** mouse **Sex:** no data  
**Strain:** no data  
**Route of admin.:** i.p.  
**Exposure period:** no data  
**Doses:** 0.2 to 2.0 ml/Kg/day

**Method:** other: see ref.  
**Year:** 1971  
**GLP:** no data  
**Test substance:** no data

**Remark:** Studies of chromosomal aberrations in bone-marrow cells.  
**Result:** Most of the induced aberrations were breaks or deletions.  
**Source:** REPSOL PETROLEO, S.A. MADRID  
 13-DEC-1996 (623)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** other  
**Route of admin.:** gavage  
**Exposure period:** no data  
**Doses:** up to 320 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was administered in sunflower-seed oil to SHR strain mice at four doses ranging from 0.001-0.2 LD50. No further details were available from the abstract.

**Result:** Benzene did not induce dominant lethal mutations. A negative result was also apparently obtained after male rats were injected intraperitoneally with 0.5 ml benzene/kg bw [approximately 440 mg/kg bw] (Lyon J.P. PhD Thesis, University of California, 1975 cited in Dean B.J. Mutation Res. 47, 75-91, 1978). There is one report of dominant lethal induction in mouse spermatogonia following intraperitoneal injection of 3000 mg/kg bw (Pavlenko G.I. et al. Toksikol. Nov. Prom. Khim. Veshchestv. 15, 30-33, 1979 cited in IPCS, 1991).

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (349) (350) (564)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** other: SHR  
**Route of admin.:** gavage  
**Exposure period:** 5 weeks  
**Doses:** up to 320 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was administered in sunflower-seed oil to SHR strain mice at four doses ranging from 0.001-0.2 LD50. No further details were available from the abstract.  
Reliability: 4 (validity could not be judged)  
Original reference in Russian  
data from abstract or data from secondary literature

**Result:** Benzene did not induce dominant lethal mutations. A negative result was also apparently obtained after male rats were injected intraperitoneally with 0.5 ml benzene/kg bw [approximately 440 mg/kg bw] (Lyon J.P. PhD Thesis, University of California, 1975 cited in Dean B.J. Mutation Res. 47, 75-91, 1978). There is one report of dominant lethal induction in mouse spermatogonia following intraperitoneal injection of 3000 mg/kg bw (Pavlenko G.I. et al. Toksikol. Nov. Prom. Khim. Veshchestv. 15, 30-33, 1979 cited in IPCS, 1991).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (350) (564)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** oral unspecified  
**Exposure period:** einmalige Applikation  
**Doses:** 1758; 3516 mg/kg

**Method:** other: nach Roehrborn und Vogel, Deutsche med.  
Wochenzeitschrift, 92, 2315-2321, (1967)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Reliability: 2 (eingeschraenkt gueltig)  
Bewertung und Dokumentation nachvollziehbar und akzeptabel

**Result:** Die Versuchs- und Kontrollgruppen bestanden aus je 20  
maennl. Tieren.  
Die behandelten Tiere wurden ca. 20 Stunden nach der  
Applikation mit 3 unbehandelten weibl. Tieren fuer die  
Dauervon 7 Tagen verpaart, danach wurden den maennl. Tieren  
7 malfuer die Dauer von je 7 Tagen je 3 weibl. Tiere  
zugesetzt.  
In der niederen Dosisgruppe zeigten die Tiere  
vorneuebergehendeinen geringfuegigen Gewichtsverlust. In der  
hohen Dosisgruppe wurde neben dem zeitweisen  
Gewichtsverlust, Zittern, Unruhe und Apathie bei den  
behandelten Tieren festgestellt. Die Konzeptionsrate war in  
beiden Dosisgruppen in der ersten Paarungswoche verringert.  
Es wurden keine Veraenderungen bezueglich des  
Mutagenitaetsindex festgestellt.

**Source:** BASF AG Ludwigshafen  
13-DEC-1996 (77)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** oral unspecified  
**Exposure period:** einmalige Applikation  
**Doses:** 1758; 3516 mg/kg

**Method:** other: nach Roehrborn und Vogel, Deutsche med.  
Wochenzeitschrift, 92, 2315-2321, (1967)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Die Versuchs- und Kontrollgruppen bestanden aus je 20  
maennl. Tieren.  
Die behandelten Tiere wurden ca. 20 Stunden nach der  
Applikation mit 3 unbehandelten weibl. Tieren fuer die  
Dauervon 7 Tagen verpaart, danach wurden den maennl. Tieren  
7 malfuer die Dauer von je 7 Tagen je 3 weibl. Tiere  
zugesetzt.  
In der niederen Dosisgruppe zeigten die Tiere  
vorneuebergehendeinen geringfuegigen Gewichtsverlust. In der  
hohen Dosisgruppe wurde neben dem zeitweisen  
Gewichtsverlust, Zittern, Unruhe und Apathie bei den  
behandelten Tieren festgestellt. Die Konzeptionsrate war in  
beiden Dosisgruppen in der ersten Paarungswoche verringert.

Es wurden keine Veraenderungen bezueglich des Mutagenitaetsindex festgestellt.

**Source:** BASF AG Ludwigshafen  
**Reliability:** (2) valid with restrictions  
 Bewertung und Dokumentation nachvollziehbar und akzeptabel  
 08-NOV-1995 (77)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** oral unspecified  
**Exposure period:** einmalige Applikation  
**Doses:** 1758; 3516 mg/kg

**Method:** other: nach Standard Protocol for the Dominant Lethal Test on Male Mice, Arch. Toxicol., 39, 173-185, (1978)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Reliability: 1 (uneingeschraenkt gueltig)  
 Guideline-aehnliche Studie nach Standard-Vorschrift

**Result:** Die Kontroll- und Versuchsgruppen bestanden aus je 45 maennlichen Tieren. Diese wurden ca. 20 Stunden nach der Applikation mit je einem unbehandelten Weibchen fuer 4 Tage verpaart. Danach wurden den gleichen maennlichen Tieren, in 11 weiteren aufeinanderfolgenden Versuchsserien von je 4 Tagen Dauer, jeweils ein neues weibl. Tier zugesetzt. In der niederen Dosisgruppe starb ein Tier, in der hohen Dosisgruppe 4 Tiere waehrend der Versuchsdauer. Bei den Tieren der hohen Dosisgruppe wurde voruebergehend eine Gewichtsabnahme festgestellt. Bis auf eine erniedrigte Konzeptionsrate im ersten Paarungsintervall in der hohen Dosisgruppe wurden keine Effekte auf die Konzeptionsraten, die durchschnittliche Zahl der Implantate, den prozentualen Anteil lebender Foeten bzw. abgestorbener Implantate festgestellt. Der Mutagenitaetsindex und Dominant-Letal-Faktor waren aufgrund der Behandlung nicht veraendert.

**Source:** BASF AG Ludwigshafen  
 13-DEC-1996 (79)

**Type:** Dominant lethal assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** oral unspecified  
**Exposure period:** einmalige Applikation  
**Doses:** 1758; 3516 mg/kg

**Method:** other: nach Standard Protocol for the Dominant Lethal Test on Male Mice, Arch. Toxicol., 39, 173-185, (1978)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Die Kontroll- und Versuchsgruppen bestanden aus je 45 maennlichen Tieren. Diese wurden ca. 20 Stunden nach der Applikation mit je einem unbehandelten Weibchen fuer 4 Tage verpaart. Danach wurden den gleichen maennlichen Tieren, in

11 weiteren aufeinanderfolgenden Versuchsserien von je 4 Tagen Dauer, jeweils ein neues weibl. Tier zugesetzt. In der niederen Dosisgruppe starb ein Tier, in der hohen Dosisgruppe 4 Tiere waehrend der Versuchsdauer. Bei den Tieren der hohen Dosisgruppe wurde voruebergehend eine Gewichtsabnahme festgestellt. Bis auf eine erniedrigte Konzeptionsrate im ersten Paarungsintervall in der hohen Dosisgruppe wurden keine Effekte auf die Konzeptionsraten, die durchschnittliche Zahl der Implantate, den prozentualen Anteil lebender Foeten bzw. abgestorbener Implantate festgestellt. Der Mutagenitaetsindex und Dominant-Letal-Faktor waren aufgrund der Behandlung nicht veraendert.

**Source:** BASF AG Ludwigshafen  
**Reliability:** (1) valid without restriction  
Guideline-aehnliche Studie nach Standard-Vorschrift  
08-NOV-1995 (79)

**Type:** Drosophila SLRL test  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** no data  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)  
**Method:** OECD Guide-line 477 "Genetic Toxicology: Sex-linked Recessive Lethal Test in Drosophila melanogaster"  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. They were then mated daily to 5 Basc virgins for 12 days to obtain 12 successive one-day broods. The progeny were pair mated separately for each brood and these pair matings were scored for the presence or absence of lethals. All suspected lethals were confirmed by testing for one more generation. Laboratory cumulative control data were used as the comparison.

**Result:** Benzene did not induce any sex-linked recessive lethal mutations.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (593)

**Type:** Drosophila SLRL test  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** no data  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)

**Method:** other: in compliance with OECD Guide-line 477  
**Year:** 1984  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. They were then mated daily to 5 Basc virgins for 12 days to obtain 12 successive one-day broods. The progeny were pair mated separately for each brood and these pair matings were scored for the presence or absence of lethals. All suspected lethals were confirmed by testing for one more generation. Laboratory cumulative control data were used as the comparison.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about test substance, one dose

**Result:** Benzene did not induce any sex-linked recessive lethal mutations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (593)

**Type:** Heritable translocation assay  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** no data  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. For the detection of heritable translocations, the treated males were individually mated to ten virgin females for 2 days to obtain one brood. Six such broods were cultured and the F1 males individually back crossed to one virgin. These pair matings were scored for brown-and-scarlet or white-eye coloured males.

**Result:** A 1-hour exposure to 88 mg/l kills approximately 80% of the exposed flies in 2 or 3 days. Benzene did not induce translocations in the spermatozoon, spermatid or spermatocyte chromosomes when compared with the controls.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (593)

**Type:** Heritable translocation assay  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** no data  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. For the detection of heritable translocations, the treated males were individually mated to ten virgin females for 2 days to obtain one brood. Six such broods were cultured and the F1 males individually back crossed to one virgin. These pair matings were scored for brown-and-scarlet or white-eye coloured males.  
Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
no data about the TS

**Result:** A 1-hour exposure to 88 mg/l kills approximately 80% of the exposed flies in 2 or 3 days. Benzene did not induce translocations in the spermatozoon, spermatid or spermatocyte chromosomes when compared with the controls.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (593)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 10.2, 25.6, 64, 160, 400, 2500 µl/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten male and ten female Swiss Lane Petter mice were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The mice were given an intraperitoneal injection of colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determinations.

**Result:** The mean numbers of polychromatic erythrocytes with micronuclei increased with dose from 22.5 mg/kg bw/day. The number of polychromatic erythrocytes was reduced at 2198 mg/kg bw/day and, therefore, the reading of smears was unsatisfactory. Male mice were more sensitive to micronucleus induction than the females. These

investigators have previously reported positive micronuclei tests in mice (Siou G. & Conan L. Cah. Notes Doc. 89, 443-444, 1977; Siou G. & Sourdeix D. ibid. 101, 531-534, 1980).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** "Chemically pure" benzene tested. (1042)  
13-DEC-1996

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** ICR  
**Route of admin.:** gavage  
**Exposure period:** 2 weeks  
**Doses:** 0, 36.6, 73.2, 146.4 mg/kg bw/day

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten mice were administered benzene in olive oil daily for 2 weeks with no treatment on days 5 and 10. Control animals received olive oil only. Blood was withdrawn to assess micronucleus frequency on days 3, 6, 9, 12 and 15. Half the animals were maintained after treatment and sampled eight times, the last sample being obtained on day 60 after treatment.

**Result:** Benzene induced significant dose- and time-dependent increases in micronucleus frequency during treatment. In the mid- and high-dosed groups, increases were noted from day 9 and in the low-dosed group, the micronucleus frequency was similar to the control levels until day 15. After day 11 post-treatment, the micronuclei levels began to decline but remained significantly higher than in the control group. In the treatment groups, erythropoiesis was dose-dependently suppressed during treatment. The levels of polychromatic erythrocytes in each dose group began to increase from day 9 and reached normal levels by day 15 of treatment.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; thiophene-free benzene tested. (943)  
13-DEC-1996

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** 5 days per week/6-8 weeks  
**Doses:** 0.03, 0.06, 0.125, 0.250, 0.5 ml/kg bw/day (converts to 26, 53, 110, 220, 440 mg/kg bw/day)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was administered in olive oil. Peripheral blood smears from the tail vein were prepared and the incidence of micronuclei scored. Micronuclei induction was also determined in bone marrow cells after a single treatment of 220 mg/kg bw/day. Control animals were treated with olive oil alone.

**Result:** No evidence of toxicity was seen in animals treated for up to 6 weeks except for decreased body weight and polychromatic/normochromatic erythrocytes in the top-dosed males. A significant increase in micronuclei in normochromatic erythrocytes was seen after 21 days in the high-dosed males and females, the females being less sensitive. A dose-response relationship was seen when treatment was for longer than 21 days. A decline of micronuclei after longer periods was also observed. The investigators thought this may be due to a selection of resistant cells, a decreased ability to metabolise benzene towards genotoxic metabolites or an increased capability in detoxification of active benzene metabolites. The frequency of micronucleated polychromatic erythrocytes obtained in bone marrow cells was increased after a single benzene dose. In splenectomized male mice treated with 220 mg/kg bw/day, micronucleated circulating normochromatic erythrocytes were increased after 21 days of treatment followed by a significant decrease after 6 weeks. No spontaneous increase was noted in the control group. Other investigators have also reported positive findings in CD1 mice (Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984; Hite M. et al. ibid. 77, 149-155, 1980; Meyne J. & Legator M.S. Envir. Mutagen. 2, 43-50, 1980).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** "Purest grade" benzene tested.  
13-DEC-1996

(69)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** ICR  
**Route of admin.:** oral unspecified  
**Exposure period:** 1 day  
**Doses:** 0, 220, 440, 880 mg/kg bw  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of five or more mice were treated with single doses of benzene alone or coadministered with pyridine, aniline or naphthalene to modify benzene's metabolism, all in olive oil. In one group, *O*-naphthoflavone was injected intraperitoneally 1 hour before benzene treatment. Some groups were induced with Aroclor-1254 or 3-methylcholanthrene. Animals were sacrificed 24 hours after benzene treatment and the numbers of micronuclei in the femoral bone marrow assessed. Urinary samples were collected to measure the benzene metabolites.

**Result:** Pyridine administered at 50 mg/kg bw and above inhibited benzene's clastogenicity when benzene was given at 880 mg/kg bw. The clastogenicity was partially blocked at this dose by 5 mg/kg bw pyridine while 0.5 mg/kg bw was ineffective. Urinary analysis showed pyridine to completely block benzene metabolism. Aniline or naphthalene coadministration enhanced the effect of benzene in the micronucleus assay. Urinary analysis in the aniline/benzene treated mice showed increased levels of hydroquinone in mice treated with 220 mg benzene/kg bw and 125 mg aniline/kg bw. As the aniline dose increased, the levels of hydroquinone and catechol decreased and the amount of phenol increased slightly. As the benzene dose increased, aniline did not affect its metabolism or clastogenicity. In the presence of naphthalene, the amounts of urinary hydroquinone and catechol were decreased. Pretreatment with *O*-naphthoflavone totally blocked both benzene's metabolism and clastogenicity when injected at high levels of 170 mg/kg bw. 3-Methylcholanthrene and phenobarbital increased the metabolism of benzene but clastogenicity was increased only by the former.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; thiophene-free benzene tested.  
13-DEC-1996

(481)

**Type:** Micronucleus assay  
**Species:** hamster **Sex:** male/female  
**Strain:** other  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The hamsters were injected intraperitoneally with colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determination.

**Result:** No dose-related increase in the numbers of polychromatic erythrocytes with micronuclei were found. In the females given 2.2 g/kg bw/day, there was a slight increase in the mean number of micronuclei but this was not seen at the higher dose or in the male animals.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** "Chemically pure" benzene tested.  
13-DEC-1996

(1042)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** single dose  
**Doses:** 880 mg/kg bw  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was administered in distilled water to male, female and, on day 13 of gestation, pregnant mice. At different periods after treatment, four animals were killed. Foetal liver and adult bone marrow cells were obtained and assessed for micronuclei. Further groups of animals were exposed to the benzene metabolites, catechol, p-benzoquinone, phenol, 1,2,4-benzenetriol, hydroquinone, o,o'-biphenol and p,p'-biphenol.

**Result:** Micronucleus induction was statistically significantly elevated in the males, females and pregnant females at each monitoring period. The males were more susceptible than the females and the virgin females more so than the pregnant females. Micronuclei were also induced in the foetal liver cells together with a high cell toxicity. All of the tested benzene metabolites induced micronuclei and, with the exception of catechol and 1,2,4-benzene-triol, produced myelotoxicity. The investigators concluded that the induction of micronuclei by benzene cannot be attributed

solely to any particular benzene metabolite tested in this study. Hydroquinone and, to a lesser extent, p-benzoquinone, catechol and o,o'-biphenol were active in inducing micronuclei and toxic effects in the foetal liver cells. p-Benzo-quinone, 1,2,4-benzenetriol and p,p'-biphenol produced toxic effects alone in the foetal cells.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (208)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** single dose  
**Doses:** 0, 880 mg/kg bw  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone.

**Result:** Benzene induced micronuclei with peak activity after 24 hours. The genotoxicity then rapidly decreased but still caused significant effects after 48 hours. Bone marrow depression increased slowly, becoming evident and significant after 42 hours and reaching a maximum after 48 hours. All of the metabolites tested induced micronuclei although phenol and hydroquinone had weak effects. Bone marrow depression occurred in the phenol, p-benzoquinone and catechol treated animals. Hydroquinone produced negligible bone marrow depression until 42 hours after treatment.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (209)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of admin.:** inhalation  
**Exposure period:** up to 16 weeks  
**Doses:** 10, 25, 100, 300, 400 ppm (converts to 0.033, 0.083, 0.33, 0.98, 1.3 mg/l)  
**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Remark:** The method of Schlegel R. & MacGregor J.T. Mutation Res. 104, 367-369, 1982 was followed. C57bl/6 mice were exposed for 9 days to 0.033, 0.083, 0.33 and 1.30 mg/l and peripheral blood smears were prepared 1 day later to analyse

the frequency of micronuclei. For the 0.98 mg/l dose level, mice were exposed for 2, 4, 8 and 16 weeks and samples were taken 3 days, 2, 4, 8 and 16 weeks following cessation of exposure.

**Result:** The micronucleus frequency was statistically significantly increased in a dose-dependent manner in normochromatic erythrocytes of mice exposed at all the 9-day levels. Similar increases were seen in the polychromatic erythrocytes of animals exposed to 0.083, 0.33 and 1.30 mg/l. When the micronucleus frequency was examined after different exposure and recovery times, the results suggested a lack of residual bone marrow damage and an alteration in the sensitivity of the animals to benzene with increasing duration of the exposure.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (1136)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** 3 days  
**Doses:** 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene diluted in corn oil. Control animals received corn oil only. Groups of five males and five females were exposed at each dose level. The animals were sacrificed 4 hours after the final dose, the femurs removed and bone marrow used for cytogenetic preparations.

**Result:** No increase in frequency of micronuclei was seen in the low-dosed females and no significant increase in the mid- and high-dosed females. In the male mice, a dose-related increase in the frequency of micronuclei was noted; the increases being significant at the two higher doses.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** >99% pure.  
13-DEC-1996 (771)

**Type:** Micronucleus assay  
**Species:** mouse  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** single dose  
**Doses:** 0, 880 mg/kg bw

**Sex:** male

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone.

**Result:** A significant increase of micronuclei was seen after 18 hours. Bone marrow depression was also noted and remained constant up to 48 hours. Phenol produced immediate genotoxic effects which were no longer significant after 42 hours. Bone marrow depression also occurred. Genotoxicity which peaked at 18 hours and was not significant after 42 hours was seen with hydroquinone. Bone marrow depression induction was rapid, peaking after 18 hours and returning to normal after 48 hours. p-Benzoquinone did not induce a significant micronucleus increase but high levels of toxicity were seen. Significant genotoxic effects were seen 24 hours after catechol treatment and bone marrow depression was evident after 18 hours.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

(209)

**Type:** Micronucleus assay  
**Species:** mouse  
**Strain:** other: Swiss ICR  
**Route of admin.:** gavage  
**Exposure period:** 2 weeks  
**Doses:** 0, 36.6, 73.2, 146.4 mg/kg bw/day

**Sex:** male

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten mice were administered benzene in olive oil daily for 2 weeks with no treatment on days 5 and 10. Control animals received olive oil only. Blood was withdrawn to assess micronucleus frequency on days 3, 6, 9, 12 and 15. Half the animals were maintained after treatment and sampled eight times, the last sample being obtained on day 60 after treatment.

Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions one sex, no positive control, no toxicity data

**Result:** Benzene induced significant dose- and time-dependent increases in micronucleus frequency during treatment. In the mid- and high-dosed groups, increases were noted from

day 9 and in the low-dosed group, the micronucleus frequency was similar to the control levels until day 15. After day 11 post-treatment, the micronuclei levels began to decline but remained significantly higher than in the control group.

In the treatment groups, erythropoiesis was dose-dependently suppressed during treatment. The levels of polychromatic erythrocytes in each dose group began to increase from day 9 and reached normal levels by day 15 of treatment.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; thiophene-free benzene tested.  
06-JAN-1997 (943)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** 5 days per week, 6-8 weeks  
**Doses:** 0.03, 0.06, 0.125, 0.250, 0.5 ml/kg bw/day (converts to 26, 53, 110, 220, 440 mg/kg bw/day)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was administered in olive oil. Peripheral blood smears from the tail vein were prepared and the incidence of micronuclei scored. Micronuclei induction was also determined in bone marrow cells after a single treatment of 220 mg/kg bw. Control animals were treated with olive oil alone.

Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions one dose, no data about PCE/NCE ratio in bone marrow experiment, no positive control, 3 mice per group

**Result:** No evidence of toxicity was seen in animals treated for up to 6 weeks except for decreased body weight and polychromatic/normochromatic erythrocytes in the top-dosed males. A significant increase in micronuclei in normochromatic erythrocytes was seen after 21 days in the high-dosed males and females, the females being less sensitive. A dose-response relationship was seen when treatment was for longer than 21 days. A decline of micronuclei after longer periods was also observed. The investigators thought this may be due to a selection of resistant cells, a decreased ability to metabolise benzene towards genotoxic metabolites or an increased capability in detoxification of active benzene metabolites. The frequency of micronucleated polychromatic erythrocytes obtained in bone marrow cells was increased after a single benzene dose.

In splenectomized male mice treated with 220 mg/kg bw/day, micronucleated circulating normochromatic erythrocytes were increased after 21 days of treatment followed by a significant decrease after 6 weeks. No spontaneous increase was noted in the control group. Other investigators have also reported positive findings in CD1 mice (Gad-El-Karim M.M. et al. Mutation Res. 135, 225-243, 1984; Hite M. et al.

ibid. 77, 149-155, 1980; Meyne J. & Legator M.S. *Envir. Mutagen.* 2, 43-50, 1980).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** "Purest grade" benzene tested.  
 06-JAN-1997 (69)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** ICR  
**Route of admin.:** other: oral application in olive oil  
**Exposure period:** once  
**Doses:** 0, 220, 440, 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of five or more mice were treated with single doses of benzene alone or coadministered with pyridine, aniline or naphthalene to modify benzene's metabolism, all in olive oil. In one group,  $\alpha$ -naphthoflavone was injected intraperitoneally 1 hour before benzene treatment. Some groups were induced with Aroclor-1254 or 3-methylcholanthrene. Animals were sacrificed 24 hours after benzene treatment and the numbers of micronuclei in the femoral bone marrow assessed. Urinary samples were collected to measure the benzene metabolites.  
 Reliability: 2 (valid with restriction)  
 Comparable to guideline study with acceptable restrictions no positive control, one sex, one sampling time, no data about PCE/NCE ratio

**Result:** Benzene induced significant dose dependent increase in micronucleus frequency.  
 Pyridine administered at 50 mg/kg bw and above inhibited benzene's clastogenicity when benzene was given at 880 mg/kg bw. The clastogenicity was partially blocked at this dose by 5 mg/kg bw pyridine while 0.5 mg/kg bw was ineffective. Urinary analysis showed pyridine to completely block benzene metabolism. Aniline or naphthalene coadministration enhanced the effect of benzene in the micronucleus assay. Urinary analysis in the aniline/benzene treated mice showed increased levels of hydroquinone in mice treated with 220 mg benzene/kg bw and 125 mg aniline/kg bw. As the aniline dose increased, the levels of hydroquinone and catechol decreased and the amount of phenol increased slightly. As the benzene dose increased, aniline did not affect its metabolism or clastogenicity. In the presence of naphthalene, the amounts of urinary hydroquinone and catechol were decreased. Pretreatment with  $\alpha$ -naphthoflavone totally blocked both benzene's metabolism and clastogenicity when injected at high levels of 170 mg/kg bw. 3-Methylcholanthrene and phenobarbital increased the metabolism of benzene but clastogenicity was increased only by the former.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; thiophene-free benzene tested.  
 06-JAN-1997 (481)

**Type:** Micronucleus assay  
**Species:** Chinese hamster **Sex:** male/female  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 2.5, 10 ml/kg bw/day (converts to 0, 2.2, 8.8 g/kg bw/day)  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten male and ten female Chinese hamsters were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The hamsters were injected intraperitoneally with colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determination. Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, one sampling time, no data about PCE/NCE ratio

**Result:** No dose-related increase in the numbers of polychromatic erythrocytes with micronuclei were found. In the females given 2.2 g/kg bw/day, there was a slight increase in the mean number of micronuclei but this was not seen at the higher dose or in the male animals.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** "Chemically pure" benzene tested.

06-JAN-1997

(1042)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** single dose  
**Doses:** 0, 880 mg/kg bw  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene was administered in distilled water to male, female and, on day 13 of gestation, pregnant mice. At different periods after treatment, four animals were killed. Foetal liver and adult bone marrow cells were obtained and assessed for micronuclei. Further groups of animals were exposed to the benzene metabolites, catechol, p-benzoquinone, phenol, 1,2,4-benzenetriol, hydroquinone, o,o'-biphenol and p,p'-biphenol. Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, no data about TS, one dose, 4 instead of 5 recommended mice/group

**Result:** Micronucleus induction was statistically significantly elevated in the males, females and pregnant females at each monitoring period. The males were more susceptible than the

females and the virgin females more so than the pregnant females. Micronuclei were also induced in the foetal liver cells together with a high cell toxicity. All of the tested benzene metabolites induced micronuclei and, with the exception of catechol and 1,2,4-benzene-triol, produced myelotoxicity. The investigators concluded that the induction of micronuclei by benzene cannot be attributed solely to any particular benzene metabolite tested in this study. Hydroquinone and, to a lesser extent, p-benzoquinone, catechol and o,o'-biphenol were active in inducing micronuclei and toxic effects in the foetal liver cells. p-Benzo-quinone, 1,2,4-benzenetriol and p,p'-biphenol produced toxic effects alone in the foetal cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (208)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** single dose  
**Doses:** 0, 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS: laboratory reagent grade

**Remark:** Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone. Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions one sex, no positive control, one dose, 4 instead of 5 recommended mice/group

**Result:** Benzene induced micronuclei with peak activity after 24 hours. The genotoxicity then rapidly decreased but still caused significant effects after 48 hours. Bone marrow depression increased slowly, becoming evident and significant after 42 hours and reaching a maximum after 48 hours. All of the metabolites tested induced micronuclei although phenol and hydroquinone had weak effects. Bone marrow depression occurred in the phenol, p-benzoquinone and catechol treated animals. Hydroquinone produced negligible bone marrow depression until 42 hours after treatment.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (209)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** other: DBA/2  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0, 10, 100, 1000 ppm (converts to 0, 0.03, 0.33, 3.26 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene concentrations were analysed hourly. Groups of five DBA/2 mice/dose were exposed. The control group consisted of ten animals exposed to air. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per animal. Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, no data about TS, one sex, one sampling time, no data about toxicity and PCE/NCE ratio

**Result:** A statistically significant, dose-related increase in the number of bone marrow polychromatic erythrocytes containing micronuclei was found. The top dose caused a 13.4-fold increase in the control level.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (338)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of admin.:** inhalation  
**Exposure period:** up to 16 weeks  
**Doses:** 10, 25, 100, 300, 400 ppm (converts to 0.033, 0.083, 0.33, 0.98, 1.3 mg/l)

**Method:** other  
**Year:** 1982  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 4 (validity cannot be judged)  
Abstract  
The method of Schlegel R. & MacGregor J.T. Mutation Res. 104, 367-369, 1982 was followed. C57bl/6 mice were exposed for 9 days to 0.033, 0.083, 0.33 and 1.30 mg/l and peripheral blood smears were prepared 1 day later to analyse the frequency of micronuclei. For the 0.98 mg/l dose level, mice were exposed for 2, 4, 8 and 16 weeks and samples were taken 3 days, 2, 4, 8 and 16 weeks following cessation of exposure.

**Result:** The micronucleus frequency was statistically significantly increased in a dose-dependent manner in normochromatic erythrocytes of mice exposed at all the 9-day levels. Similar increases were seen in the polychromatic erythrocytes

of animals exposed to 0.083, 0.33 and 1.30 mg/l. When the micronucleus frequency was examined after different exposure and recovery times, the results suggested a lack of residual bone marrow damage and an alteration in the sensitivity of the animals to benzene with increasing duration of the exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn (1135)  
06-JAN-1997

**Type:** Micronucleus assay  
**Species:** rat **Sex:** male  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions no positive control, no data about TS (but concurrent analysis), one sex, one sampling time, no data about toxicity and PCE/NCE ratio  
The top two benzene concentrations were analysed hourly, the other concentrations being analysed two or three times per hour. The control group consisted of ten to 20 animals exposed to air. Groups of five animals/dose were exposed to benzene. Femoral bone marrow was prepared and stained 18 hours after exposure by a modification of the technique by Schmid W. In: Chemical Mutagens: Principles and Methods for their Detection. Vol.4. p.31-53. Edited by A. Hollaender. Plenum Press, New York, 1976. Four slides were prepared per animal.

**Result:** Benzene induced statistically significant, dose-dependent increases in the numbers of polychromatic erythrocytes containing micronuclei at concentrations of 0.003 to 0.098 mg/l. There was no observable effect at the lower two doses.

**Source:** Deutsche Shell Chemie GmbH Eschborn (338)  
06-JAN-1997

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** single dose  
**Doses:** 0, 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** other TS: laboratory reagent grade

**Remark:** Groups of four animals were used. Benzene was administered in olive oil. Control animals were treated with olive oil. Animals were killed and bone marrow smears prepared at various times 0-48 hours after treatment. Further groups of animals were similarly exposed to the benzene metabolites, catechol, p-benzoquinone, phenol and hydroquinone. Reliability: 2 (valid with restriction) Comparable to guideline study with acceptable restrictions no positive control, one sex, 4 instead of 5 recommended animals per group

**Result:** A significant increase of micronuclei was seen after 18 hours. Bone marrow depression was also noted and remained constant up to 48 hours. Phenol produced immediate genotoxic effects which were no longer significant after 42 hours. Bone marrow depression also occurred. Genotoxicity which peaked at 18 hours and was not significant after 42 hours was seen with hydroquinone. Bone marrow depression induction was rapid, peaking after 18 hours and returning to normal after 48 hours. p-Benzoquinone did not induce a significant micronucleus increase but high levels of toxicity were seen. Significant genotoxic effects were seen 24 hours after catechol treatment and bone marrow depression was evident after 18 hours.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (209)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** Daily for 2 consecutive days; sacrificed at 6 hours to 16 days post-dose  
**Doses:** 0.0625, 0.125, 0.25, 0.5, 1.0 or 2.0 ml/kg/day. Positive control: 0.26 mg/ml methyl methanesulfonate (i.p.). Negative control: corn oil (p.o.).

**Method:** other: Hite, H. et al.  
**Year:** 1980  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Mice (4-5/sex/benzene group) were sacrificed 6, 18, 24 or 48hours, or 5, 9 or 16 days post exposure. Bone marrow was evaluated for micronuclei formation. Approximately 3000 polychromatic (PCE) and normochromatic (NCE) erythrocytes were evaluated per animal. Responses were similar for both

sexes. The number of micronuclei in NCE's showed no relationship to treatment. A significant increase in micronucleated PCE's was observed at 6 hours, at doses greater than or equal to 0.25 ml/kg/day; and at 18 or 24 hours in mice exposed at 0.125 mg/kg/day or higher benzene. However, in a second experiment, mice exposed to 0.0625, 0.125, or 0.25 mg/kg/day benzene failed to show increased micronuclei formation at the 24 hour sacrifice. Mice exposed at concentrations greater than or equal to 0.125 mg/kg/day and sacrificed 5 days post exposure failed to show a significant increase in micronucleated PCE's. By Days 9 or 16 of sacrifice, mice exposed at the highest concentrations (0.5, 1.0 or 2.0 mg/kg/day) exhibited micronuclei values that were similar to negative controls.

**Result:** Results: positive  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (504)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** 5 days/week, for 42 days  
**Doses:** 0.00, 0.03, 0.06, 0.125, 0.250, 0.500 ml/kg body weight

**Method:** other: T. MacGregor et al.  
**Year:** 1980  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** The study methodology is based on the hypothesis that, unlike man and the rat, mouse damaged erythrocytes are not eliminated by the spleen. Both normal and splenectomized mice were administered benzene in corn oil by oral gavage (3/sex/dose group). Peripheral blood was evaluated weekly for micronucleus formation. A total of 3000 normochromatic erythrocytes were evaluated per animal. A slight decrease in body weight and decreased polychromatic (PCE): NCE ratio was evident in high dose males. A significant increase in micronuclei formation was only observed in male and female mice (with intact spleens) after Day 21 at the highest dose groups (0.250, 0.50 ml/kg body weight). A dose-response was evident following exposure Day 21, reaching a steady state after Day 28 in females and Day 42 in males (depending on dose level). However, females were less sensitive to micronuclei formation than were their male counterparts. An increase in micronuclei formation was also observed in splenectomized mice exposed to 0.25 ml/kg benzene after Day 21, followed by a significant decrease in micronuclei at Day 42.

**Result:** Results: positive  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (69) (718)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** inhalation  
**Exposure period:** Continuous exposure for 10 days  
**Doses:** 0, 1, 10, 14, 21, 50, 95 ppm

**Method:** other: Toft et al.  
**Year:** 1982  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Continuous exposure of mice (5/group) to benzene for a period of 4 - 10 days at levels as low as 21 ppm significantly affected the number of nucleated cells/tibia, the number of colony forming granulopoietic stem cells (CFU-C)/tibia and the number micronucleated polychromatic erythrocytes (PCE's) in the bone marrow. At lower levels (10 and 1 ppm) no reproducible effects on cellularity or CFU-C/tibia could be demonstrated even after 8 weeks of exposure. However, at 14 ppm the frequency of micronucleated PCE's was slightly elevated after only 1 week of exposure but did not continue to increase with greater exposure periods.

**Result:** Results: positive  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1142)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** NMRI  
**Route of admin.:** inhalation  
**Exposure period:** 8 hours/day, 5 days/week for 2 weeks  
**Doses:** 0, 1.0, 10.5, 21, 50, 95, and 107 ppm

**Method:** other: Toft et al.  
**Year:** 1982  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Intermittent exposure of mice (5 males/group) to benzene resulted in a significant decrease in the number of colony forming granulopoietic stem cells (CFU-C)/tibia and a significant increase in micronuclei frequency compared to controls at concentrations exceeding 10.5 ppm. The number of nucleated cells/tibia was significantly different from controls at concentrations exceeding 21 ppm.

**Result:** Results: measurable toxicity  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1142)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of admin.:** i.p.  
**Exposure period:** single treatment  
**Doses:** 0 - 120 mg/kg

**Method:** other: nonguideline  
**Year:** 1994  
**GLP:** no data  
**Test substance:** other TS: hydroquinone, cathecol, phenol

**Result:** Three benzene metabolites, hydroquinone (HQ), cathecol (CAT) and phenol (PHE) were studied to define their possible interaction in inducing micronuclei (Mn) in mouse bone marrow polychromatic erythrocytes (PCEs). HQ and CAT, administered separately, induced Mn while PHE showed no genotoxic effects. Binary and ternary mixtures of two or three metabolites gave different results, causing considerable increase or decrease in MN induction. HQ and PHE, in binary mixtures, as well as PHE and CAT, increased Mn synergistically, while HQ and CAT interacted negatively. The genotoxicity of ternary mixtures was mainly the consequence of two metabolites: HQ and CAT. The maximal effect obtained is far below the induction of Mn consequent to benzene treatment. These data suggest that toxic and genotoxic effects of benzene alone could be the result of more complex interactions among these and other metabolites.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (746)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: Swiss Lane Petter  
**Route of admin.:** gavage  
**Exposure period:** 24 hours  
**Doses:** 0, 10.2, 25.6, 64, 160, 400, 2500 ul/kg bw/day (converts to 0, 8.96, 22.5, 56.3, 140, 352, 2198 mg/kg bw/day)

**Method:** other: in compliance with OECD Guideline 474  
**Year:** 1985  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Groups of ten male and ten female Swiss Lane Petter mice were administered benzene in peanut oil. Control animals received peanut oil. The animals were given two administrations, of the same benzene dose, at 24-hour intervals. The mice were given an intraperitoneal injection of colchicine 3 hours after the second benzene dose and were killed 3 hours later. Bone marrow cells from the femur were collected and prepared for micronucleus determinations. Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no positive control, single sampling time

**Result:** The mean numbers of polychromatic erythrocytes with micronuclei increased significantly with dose from 56.3

mg/kg bw/day.

The number of polychromatic erythrocytes was reduced at 2198 mg/kg bw/day and, therefore, the reading of smears was unsatisfactory. Male mice were more sensitive to micronucleus induction than the females. These investigators have previously reported positive micronuclei tests in mice (Siou G. & Conan L. Cah. Notes Doc. 89, 443-444, 1977; Siou G. & Sourdeix D. ibid. 101, 531-534, 1980).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** "Chemically pure" benzene tested.  
06-JAN-1997 (1042)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: Swiss CD-1  
**Route of admin.:** gavage  
**Exposure period:** 3 days  
**Doses:** 0, 0.1, 0.5, 1 ml/kg bw/day (converts to 0, 88, 440, 880 mg/kg bw/day)

**Method:** other: nearly in compliance with OECD Guideline 474  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene diluted in corn oil. Control animals received corn oil only. Groups of five males and five females were exposed at each dose level. The animals were sacrificed 4 hours after the final dose (2 h after colchicine treatment, assay combined with metaphase analysis), the femurs removed and bone marrow used for cytogenetic preparations. 5 male and 5 female mice per group.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
**Result:** no positive control, no toxicity data, one sampling time  
Significant increase in frequency of micronuclei was seen in the in the mid- and high-dosed male and female mice.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** >99% pure.  
06-JAN-1997 (771)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** unspecified  
**Exposure period:** 0, 15, 30, 60, 90 days  
**Doses:** 1, 10, 30, 300 ppm

**Method:** other: see ref.  
**Year:** 1985  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Exposure to 3000 ppm benzene caused a significant increase in micronucleated polychromatic and monochromatic erythrocytes in male and female mice at all simple times.  
**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(358)

**Type:** Micronucleus assay**Species:** mouse**Sex:****Method:**

## ANIMALS AND TREATMENTS

Groups of male and female mEH -/- mice (deficient in epoxide hydrolase, confirmed by genotyping) and 129/sv mice (described as background genotype) were exposed to 0, 10, 50 or 100 ppm benzene vapour, 6 hr/d, 5 d/wk for 2 wk. The animals were 12 wk old at the start of the exposures. The concentration of benzene within the chambers was determined at least 3 times per exposure using a MIRAN 1A infrared spectrophotometer. Tissues were collected at necropsy, 5 hr after termination of the final exposure, under pentobarbital anaesthesia. Blood was collected by cardiac puncture (EDTA) for total white cell counts. Additional blood was collected for micronucleus analyses. The femur and humerus were samples, the marrow removed and used for isolation of RNA (femur) or determination of total cell counts (humerus).

## DETERMINATION OF MICRONUCLEI IN BLOOD

The frequency or percentage of micronucleated (MN) reticulocytes (RET) and normochromatic erythrocytes (NCE) in blood was determined using flow cytometry (Prototype Microflow Mouse Micronucleus Analysis Kit, Litron Labs, Rochester, NY) after incubation with transferrin receptor-conjugated antibody, RNase and propidium iodide. MN were defined as propidium iodide positive cells, RET were identified as transferrin positive cells, and NCE as transferrin negative cells.

## REAL-TIME EXPRESSION OF p21 mRNA

Total bone marrow RNA was isolated using a proprietary kit (Qiagen RNAeasy Kit), and p21 mRNA present in reverse transcriptase-PCR preparations quantified using an ABI 7700 Prism Sequence Detection System.

Comment: p21 is a cyclin-dependent kinase inhibitor involved in p53-induced cell cycle arrest, and is increased after benzene treatment.

## DETERMINATION OF CYP2E1 ACTIVITY AND CONTENT

Liver microsomes were prepared from untreated (naïve) male and female mEH -/- and 129/sv mice and stored frozen (-80 degrees C) until use. p-Nitrophenol hydroxylase activity (PNPH, mediated by CYP2E1) was determined spectrophotometrically by following formation of p-nitrocatechol. CYP2E1 was also quantified using a primary polyclonal rat antibody and visualised (chemiluminescence) using antigoat horseradish peroxidase.

## STATISTICAL METHODS

Three way ANOVA was used to investigate the effect of gender, genotype and exposure level, with significant interactions analysed further using Tukey's multiple comparison procedure. A Pearson correlation coefficient was

**Result:**

calculated for MN RET and p21 mRNA.

**CYP2E1 ACTIVITY AND CONTENT**

PNPH in liver microsomes from male 129/Sv mice (2.03 nmol/min/mg) was approx. 50% greater than that present in male mEH -/- mice (1.33 nmol/min/mg;  $P < 0.05$ ), however the authors considered that this relatively minor increase would have had negligible impact on cytochrome P-450-mediated benzene metabolism. PNPH in females was comparable between the genotypes, while there were no sex- or genotype differences in CYP2E1 protein content.

**HAEMATOTOXICITY AND MYELOTXICITY**

White cell counts in peripheral blood were significantly decreased by approx. 50% (indicative of haematotoxicity) in male 129/Sv mice exposed to 100 ppm benzene compared to male mEH -/- mice, but there were no differences in the lower exposure groups. Significant myelotoxicity was present in male 129/Sv mice, with statistically significant decreases in total bone marrow cells relative to mEH -/- males after exposure to 50 ppm (-35%) or 100 ppm (-50%) benzene. No significant haematotoxicity or myelotoxicity was observed in either genotype of female mice, although total bone marrow cells were increased 25-30% in mEH -/- females exposed to 50 ppm or 100 ppm benzene relative to controls.

**MICRONUCLEI IN PERIPHERAL BLOOD CELLS**

MN RET and MN NCE were significantly increased 14-fold and 2.7-fold, respectively, in male 129/Sv mice exposed to 100 ppm benzene relative to controls, however male mEH -/- exposed under similar conditions showed only minor changes (20-70% increase). MN RET was significantly increased 40-70% in female 129/Sv and female mEH -/- mice, respectively, exposed to 100 ppm benzene whereas MN PCE were unchanged.

There was a clear dose response in MN RET in both sexes of 129/Sv mice and in female mEH -/- mice exposed to 0, 10, 50 or 100 ppm benzene vapour (significant in high dose animals only, as above). A dose response relationship in MN NCE was seen only in male 129/Sv mice (significance as described above).

**p21 EXPRESSION**

Expression of p21 mRNA was increased significantly in male 129/Sv mice exposed to 50 ppm (3-fold) or 100 ppm (10-fold) benzene relative to controls, and in females exposed to 100 ppm (4-fold). Expression of p21 mRNA was unaltered in exposed mEH -/- mice of both sexes.

Significant genotype differences in expression of p21 mRNA were apparent for male mice exposed to 50 or 100 ppm benzene (greater in 129/Sv, as above), whereas no significant differences were apparent between females of the two genotypes, irrespective of exposure concentration.

Expression of p21 mRNA correlated significantly with MN RET ( $r = 0.909$ ;  $P < 0.0001$ ).

**Source:**

A.K. Mallett Surrey

**Conclusion:** This study demonstrates a role for microsomal epoxide hydrolase with respect to benzene toxicity in mice, with decreased toxicity in animals with a deficient genotype. Male mEH -/- mice (deficient in microsomal epoxide hydrolase) were unresponsive whereas male 129/Sv mice developed haematotoxicity, myelotoxicity and genotoxicity. Female mice of both genotypes exhibited little response to benzene. Expression of p21 mRNA was elevated in male 129/Sv mice, and to a lesser degree in females, while no changes were seen in mEH -/- mice of either sex. The authors conclude that microsomal epoxide hydrolase appears to be critical in benzene-induced toxicity in male, but not female, mice.

17-MAR-2004

(83)

**Type:** Micronucleus assay  
**Species:** mouse **Sex:**

**Method:** ANIMALS AND TREATMENTS  
Groups of male and female NQO1 -/- mice (deficient in NAD(P)H: quinone oxidoreductase-1, confirmed by genotyping) and 129/sv mice (wild type, NQO1 +/+) were exposed to 0, 10, 50 or 100 ppm benzene vapour, 6 hr/d, 5 d/wk for 2 wk. The animals were 12 wk old at the start of the exposures. The concentration of benzene within the chambers was determined at least times per exposure using a MIRAN 1A infrared spectrophotometer. Tissues were collected at necropsy, 5 hr after termination of the final exposure, under pentobarbital anaesthesia. Blood was collected by cardiac puncture (EDTA) for total white cell counts. Additional blood was collected for micronucleus analyses (see below) The femur and humerus were sampled, the marrow removed and used for isolation of RNA (femur) or determination of total cell counts (humerus).

#### HISTOPATHOLOGY

Thymus, sternum, liver, lymph nodes and lungs were preserved, processed and stained (H&E) for subsequent histopathological evaluation (the sternum after decalcification). Bone marrow cellularity was assessed microscopically in standardised regions of mid-sternobreal marrow cavities.

#### DETERMINATION OF MICRONUCLEI IN BLOOD

The frequency or percentage of micronucleated (MN) reticulocytes (RET) and normochromatic erythrocytes (NCE) in blood was determined using flow cytometry (Prototype Microflow Mouse Micronucleus Analysis Kit, Litron Labs, Rochester, NY) after incubation with transferrin receptor-conjugated antibody, RNase and propidium iodide. MN were defined as propidium iodide positive cells, RET were identified as transferrin positive cells, and NCE as transferrin negative cells.

#### REAL-TIME EXPRESSION OF p21 mRNA

Total bone marrow RNA was isolated using a proprietary kit (Qiagen RNeasy Kit) and p21 mRNA present in reverse

transcriptase-PCR preparations quantified using an ABI 7700 Prism Sequence Detection System. (p21 is a cyclin-dependent kinase inhibitor involved in p53-induced cell cycle arrest, and is increased after benzene treatment.)

#### DETERMINATION OF CYP2E1 ACTIVITY AND CONTENT

Liver microsomes were prepared from untreated (naïve) male and female mEH -/- and 129/sv mice and stored frozen (-80 degrees C) until use. p-Nitrophenol hydroxylase activity (PNPH, mediated by CYP2E1) was determined spectrophotometrically by following formation of p-nitrocatechol. CYP2E1 was also quantified using a primary polyclonal rat antibody and visualised (chemiluminescence) using antigoat horseradish peroxidase.

#### STATISTICAL METHODS

Three way ANOVA was used to investigate the effect of gender, genotype and exposure level, with significant interactions analysed further using Tukey's multiple comparison procedure. A Pearson correlation coefficient was calculated for MN RET and p21 mRNA.

#### Result:

#### CYP2E1 ACTIVITY AND CONTENT

PNPH activity in liver microsomes was significantly lower in NQO1 -/- mice (approx. 1.2-1.3 nmol/min/mg; both sexes) in comparison 129/Sv (NQO1 +/+) mice (1.8-2.1 nmol/min/mg; females and males, respectively). In contrast, CYP2E1 protein expression was comparable in males and females of both genotypes. The authors considered that these relatively minor differences CYP2E1 expression would have negligible impact on cytochrome P-450-mediated benzene metabolism in the two genotypes.

#### HAEMATOTOXICITY

Male NQO1 -/- mice exhibited significant haematotoxicity at a lower benzene exposure (50 ppm) than male 129/Sv (NQO1 +/+) mice (total white cells decreased approx. 60% or 30%, respectively, relative to control). At 100 ppm, both genotypes exhibited haematotoxicity compared to unexposed controls (total white cells decreased approx. 80%) but did not differ from one another. Female NQO1 -/- mice were also more sensitive to benzene at 50 or 100 ppm (decreased 50-60% relative to control) than 129/Sv (NQO1 +/+) mice (decreased 20-25% relative to control).

#### HISTOPATHOLOGICAL CHANGES

Bone marrow hypoplasia in sternebrae was increased in male NQO1 -/- and male 129/Sv (NQO1 +/+) mice, and female NQO1 -/- mice, exposed to 100 ppm benzene (not lower exposure groups). There was no discernable effect on specific cell lines present in bone marrow. No treatment-related histopathological changes were present in the other tissues that were subject to microscopic evaluation.

#### MICRONUCLEI IN PERIPHERAL BLOOD CELLS

MN RET increased in an exposure-related manner in males of both genotypes, with an approx. 3-4 fold increase relative to controls after exposure to 50 ppm benzene, and a 11-13

fold increase after exposure to 100 ppm ( $P < 0.05$ ) MN NCE were also increased, with an approx. doubling in both genotypes after exposure to 100 ppm benzene vapour ( $P < 0.05$ ). (Authors note that difference in extent of response between RET and NCE may reflect limited maturation of RET to NCE during the 2wk of the study.)

The occurrence of MN RET was increased significantly ( $P < 0.05$ ) in female NQO1 -/- mice after exposure to 50 ppm (approx. 5 fold) or 100 ppm benzene (approx. 9 fold), whereas only a moderate increase (3 fold; non-significant) was found in NQO1 +/+ at 100 ppm. Levels of MN NCE in NQO1 -/- mice exposed to 100 ppm benzene were about double that of the unexposed controls (non-significant), whereas no exposure-related changes were present in NQO1 +/+ females.

MN responses in RET and NCE from male NQO1 +/+ mice were much greater than those recorded in NQO1 +/+ females.

#### p21 EXPRESSION

Expression of p21 mRNA in bone marrow was increased in a dose related manner after exposure to benzene. Males of both genotypes exhibited a  $>10$  increase after 2 wk exposure to 100 ppm benzene, whereas female NQO1 -/- females showed an approx. 9 fold increase with a more modest (approx. 4 fold) increase in female NQO1 +/+ mice.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

This study demonstrates a role for NQO1 with respect to benzene toxicity in mice, with greater toxicity in animals with a deficient genotype. The results indicate that NQO1 detoxifies benzene metabolites responsible for genotoxicity and haematotoxicity in female mice, whereas in males it is critical only for detoxifying metabolites involved in haematotoxicity.

17-APR-2004

(84)

**Type:**

Micronucleus assay

**Species:**

human

**Sex:**

**Method:**

#### SUBJECTS AND METHODS

Sixty-two volunteers (32 men, 30 women; mean subject age 34.7 yr) with possible occupational exposure to benzene were recruited from the Bologna region of Italy; 15 subjects were exposed to vehicle exhaust emissions (traffic wardens) while the remainder (47) worked in a university analytical laboratory with occasional benzene exposure. All subjects underwent a clinical examination, and characteristics of possible relevance to the study (including ingestion of foods containing sorbitol) recorded. None of the subjects had a history of occupational or therapeutic exposure to ionising radiation.

#### ASSESSMENT OF BENZENE EXPOSURE AND ANALYSIS

Benzene exposure was determined using biological monitoring and passive sampling.

For biological monitoring, a urine sample was collected 4 hr into the work shift and analysed for unchanged benzene (headspace GC, limit of detection 0.05 ug/l) and t,t-muconic acid (t,t-MA; HPLC with UV detection, limit of detection 10 ug/l).

Airborne exposure of the traffic wardens to benzene was determined using passive samplers, worn for 6 hr during a work shift. Exposure of laboratory staff was estimated from passive area monitoring (2 or 3 devices placed in the laboratory throughout an 8 hr work shift). Samples were analysed using GC-MS.

#### DETERMINATION OF MICRONUCLEI (MN)

Peripheral lymphocytes were isolated from heparinised blood by gradient centrifugation and grown in culture for 72 hr (1% phytohaemagglutinin) prior to addition of cytochalasin B. Preparations were stained with May Grunwald-Giemsa, and 2000 binucleate lymphocytes per subject scored blind. Cell cycle parameters (number of nuclei) were evaluated on 1000 cells and the nuclear division index (NDI; mean number of nuclei/cell) calculated.

#### STATISTICAL METHODS

Results were analysed using Wilcoxon rank-sum and Kruskal-Wallis tests. Multiple regression (Ramsey omitted variable test; Cook-Weisberg heteroscedasticity test) of transformed data (Box-Cox method) was used to test possible associations between the presence of micronuclei in blood with benzene exposure and environmental factors.

#### Result:

#### EXPOSURE ANALYSES

The following mean exposures (range in parentheses) were obtained for the overall study population:

Airborne benzene: 4.37 ppb (0-14.30)\*  
Urinary benzene: 0.66 ug/l (0-4.77)  
Urinary t,t-MA: 106 ug/l (7-721)  
\* personal and environmental samples combined

Following Box-Cox transformation, no significant correlation was found between benzene in air and benzene in urine ( $R^2 = 0.09$ ). Comment: The authors note that this was not unexpected since benzene uptake is only a fraction of the total external dose.

Urinary benzene and t,t-MA for the total population showed a moderate correlation ( $R^2 = 0.37$ ).

Urinary benzene levels for laboratory workers (0.79 ug/l) was significantly greater ( $P = 0.018$ ) than for traffic wardens (0.22 ug/l). A similar significant ( $P = 0.001$ ) difference was also present for t,t-MA (126 versus 46 ug/l, respectively). There was no apparent difference in airborne exposure between the laboratory workers (4.1 ppb; environmental monitoring) and the traffic wardens (5.1 ppb; personal exposure).

## MICRONUCLEI IN BLOOD

Traffic wardens (4.70 per 1000 cells) and laboratory workers (5.76 per 1000 cells) had similar MN frequencies.

There was no significant association between MN frequency and any of the air or urinary variables measured in the study.

Moderate-heavy smokers (7.31/1000 cells) had a higher number of micronuclei than light smokers (4.67/1000 cells) and non-smokers (5.35/1000 cells) (differences not statistically significant). Age and gender were without apparent effect on induction of MN.

**Source:** A.K. Mallett Surrey

**Conclusion:** This study found no evidence that benzene exposures in the range 0-14 ppb had any detectable effect on the induction of lymphocytes in peripheral lymphocytes from occupationally exposed workers.

31-MAR-2004

(1218)

**Type:** Sister chromatid exchange assay  
**Species:** mouse **Sex:** male/female  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 4 hours  
**Doses:** 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six males and six females. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrows flushed out and SCE frequency assessed.

**Result:** Benzene significantly increased the level of SCEs in bone marrow cells when compared with the control group. There was no significant difference between the treated males and females. Phenobarbital pretreatment enhanced the benzene-increased SCE frequency in female but not male mice.

**Source:** BP Chemicals Ltd LONDON

13-DEC-1996

(563) (821) (1134) (1137) (1138)

**Type:** Sister chromatid exchange assay  
**Species:** mouse **Sex:** male/female  
**Strain:** other: DBA/2  
**Route of admin.:** inhalation  
**Exposure period:** 4 hours  
**Doses:** 0, 3130 +/-170 ppm (converts to 0, 10.2 +/-0.55 mg/l)  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of ten male and eleven female DBA/2 mice were exposed to benzene. The control group consisted of six males and six females. A further group of six males and seven females were injected intraperitoneally with sodium phenobarbital, twice daily for 3 days prior to benzene exposure. Benzene concentration was measured at half-hour intervals. Control and exposed mice were treated with bromodeoxyuridine 1 hour after exposure and colcemid was injected intravenously 2 hours before termination. The animals were killed, femurs removed, marrows flushed out and SCE frequency assessed. Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
no positive control, one sampling time, single dose (exposure period)

**Result:** Benzene significantly increased the level of SCEs in bone marrow cells when compared with the control group. There was no significant difference between the treated males and females. Phenobarbital pretreatment enhanced the benzene-increased SCE frequency in female but not male mice.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1133) (1137) (1138)

**Type:** Sister chromatid exchange assay  
**Species:** mouse **Sex:** male  
**Strain:** other: DBA/2  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0, 10, 100, 1000 ppm (converts to 0, 0.033, 0.33, 3.26 mg/l)  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Each dose group consisted of five DBA/2 mice and the control group of ten mice, exposed to room air. Exposure chamber atmospheres were analysed hourly. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured. 5-Bromo-2'-deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 60 hours. Two or three slides were prepared per animal for SCE analysis. Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

**Result:** no positive control, no data about the TS ;  
Benzene induced significant, dose-related increases in the SCE frequencies at all the dose levels. A significant dose-related decrease in the mitotic activity was seen but there were no significant effects on leucocyte counts.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (338)

**Type:** Sister chromatid exchange assay  
**Species:** rat **Sex:** male  
**Strain:** Sprague-Dawley  
**Route of admin.:** inhalation  
**Exposure period:** 6 hours  
**Doses:** 0, 0.1, 0.3, 1, 3, 10, 30 ppm (converts to 0, 0.0003, 0.001, 0.003, 0.01, 0.033, 0.098 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Each dose group consisted of five animals and the control groups of ten or twenty mice, exposed to room air. Exposure chamber atmospheres were analysed hourly for the top two benzene concentrations and two to three times per hour for the other doses. Blood was withdrawn 18 hours after benzene exposure and lymphocytes were cultured. 5-Bromo-2'-deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 52 hours. Two or three slides were prepared per animal for SCE analysis. Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail

**Result:** no positive control, no data about the TS  
Benzene induced significant dose-dependent increases in the SCE frequency at 0.01 mg/l and above. The significance of the effect seen at 0.003 mg/l was borderline and dependent on the statistical test chosen, although the SCE frequency falls in line between the SCE frequencies observed at 0.001 and 0.01 mg/l and is higher in all exposed rats when compared with the controls. Benzene concentrations of 0.0003 and 0.001 mg/l did not significantly affect the SCE frequency. Mitotic activity was significantly decreased at 0.01 and 0.098 mg/l but not at 0.033 mg/l. No significant effect was seen on leucocyte count.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (338)

**Type:** Sister chromatid exchange assay  
**Species:** human **Sex:** female  
**Route of admin.:** inhalation  
**Exposure period:** at least eight years  
**Doses:** <5 ppm

**Method:** other: nonguideline  
**Year:** 1992  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** DNA strand breakage as measured by alkaline elutron was alsoassessed.

**Result:** Peripheral lymphocyte DNA damage as measured by the method of alkaline filter elution and the frequency of sister chromatid exchange (SCE) in lymphocytes was investigated for a group of 20 female workers of a shoemaking plant who were exposed to benzene and toluene, primarily below 5 and 100 ppm respectively; the results were compared with those from a control group. In the female workers significantly raised ( $P < 0.05$ ) SCE values were found. The relative DNA elution rate through polycarbonate filters was significantly increased ( $P < 0.001$ ). The elution rate through polyvinylidene fluoride (HVLP) filters showed a tendency to increase ( $P = 0.052$ ), which must be interpreted as indicating increased DNA strand breakage. The SCE rates of the female workers were significantly correlated ( $P < 0.01$ ) with the relative DNA elution rate through HVLP filters. There was no correlation with the actual benzene and toluene uptake measured by regional air monitoring.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (902)

**Type:** Sister chromatid exchange assay  
**Species:** mouse **Sex:** male/female  
**Strain:** DBA  
**Route of admin.:** inhalation  
**Exposure period:** 4 hour  
**Doses:** 3100 ppm

**Method:** other: nonguideline  
**Year:** 1980  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Exposure of adult male and female DBA/2 mice to 3100 ppm benzene for 4 hr significantly increased the frequency of sister chromatid exchanges in bone marrow cells of both sexes, inhibited marrow cellular proliferation (but only in male mice), and did not significantly increase the frequency of chromosomal aberrations in either sex. Phenobarbital pretreatment synergistically interacted with benzene exposure to further increase sister chromatid exchanges in female mice, induce greater inhibition of cellular proliferation in male mice, and induce a significant level of chromatid-type chromosomal aberrations in both sexes. The differences in both the type and

magnitude of the response of bone marrow cellular populations, as determined by different cytogenetic end points in male and female mice exposed to benzene or to phenobarbital and benzene, suggest not only that a metabolite of benzene is responsible for the observed effects, but that different metabolites may be involved in different end points.

**Source:** Deutsche Shell Chemie GmbH Eschborn (1139)  
06-JAN-1997

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male/female  
**Strain:** other  
**Route of admin.:** oral feed  
**Exposure period:** up to 96 hours  
**Doses:** 0.88, 4.4, 8.8 mg/ml

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. Benzene was administered to mei-9 and +/mei-9 strain Drosophila as a solution in 5% Tween 80 and 5% ethanol. Larvae aged 48 or 72 hours were exposed for 2 hours to benzene concentrations of 4.4 and 0.88 mg/ml respectively. In some cases, larvae were treated during the entire larval life (96 hours) to 4.4 or 8.8 mg/ml. After exposure, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and the frequency and size of the spots were recorded. Control experiments were conducted by exposing larvae of the same age as those in the treatment groups to the 5% Tween 80 and 5% ethanol vehicle for the same length of time as the corresponding treated larvae.

**Result:** A benzene concentration of 8.8 mg/ml was lethal and 4.4 mg/ml toxic when given for the entire larval life. A significant increase in the frequency of small single spots and/or total single spots was seen in the acute and chronic studies in the excision-repair-defective larvae (mei-9 strain). The acute result was only marginally positive. No such effect was seen when benzene was given to excision-repair-proficient larvae (+/mei-9 strain).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure. (1284)  
13-DEC-1996

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** other  
**Route of admin.:** oral feed  
**Exposure period:** 96 hours  
**Doses:** 0, 0.5, 1, 2 % (converts to 0, 4.4, 8.8, 17.6 mg/ml)  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in 40% ethanol. Male and female C(1)DX,yf/Y/sc z w+ mei-9a Drosophila were mated for 4-5 days and allowed to ovipost for 24 hours. The resultant eggs were allowed to develop for 24 hour and the newly hatched larvae exposed to benzene or the vehicle as the control. Benzene was added (1 ml) to the culture medium and the larvae were allowed to develop throughout the larval period (roughly 96 hours). Eyes of adult males were examined for colour mutations.

**Result:** No significant increase in mutation frequency over the control levels was seen. Benzene, however, caused a dose-dependent toxic effect on the survival of the male larvae, with 50% survival occurring at the top dose. A similar study using the genotype sc z w+ and benzene concentrations of 8.8 and 17.6 mg/ml reported similar decreases in survival and a lack of mutagenic effect (Nylander P.-O. et al. Mutation Res. 57, 163-167, 1978).

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996

(380)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** female  
**Strain:** other  
**Route of admin.:** oral feed  
**Exposure period:** 10-11 days  
**Doses:** 156 mg/l  
**Method:** other  
**Year:** 1957  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The method of Becker H.J. Z. Indukt. Abstammungs-Vererbungslehre 88, 333-373, 1957 was followed. Benzene was prepared in a mixture of 3% ethanol and 1% Tween 60 before being added to the food. Virgin females of the genotype wcosn2; se h were crossed with w; se h males and allowed to lay eggs for a period of 4 days on the food supplemented with benzene. The control larvae were exposed to the solvent. The developing culture was incubated for 10-11 days and the hatching females were scored for genetic changes which produced w clones or wco//w twin spots in their eyes.

**Result:** No increase in the number of mosaic twin spots was observed. However, the numbers of mosaic single spots and twin and

single spots were significantly increased. The authors regarded these as inconclusive as more data are needed for their final classification.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996 (1219)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male/female  
**Strain:** other  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 0.05, 0.2, 0.3 ml/1150ml (converts to 0, 38, 153, 229 mg/l)

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. mwh+/+flr and mei-9 strain larvae were exposed at an age of 48 or 72 hours. They were placed in a 1150 ml airtight container and benzene was injected and allowed to volatilize to give the required concentration. After treatment, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and frequency and size of the spots were recorded. Control groups were exposed to air at the same age and for the same length of time as the larvae in the treatment groups.

**Result:** An increase in the frequency of small single spots and/or total single spots was seen in excision-repair-proficient larvae (mwh+/+flr) exposed to 229 mg/l but not 153 mg/l and in excision-repair-defective larvae (mei-9) exposed to 38 mg/l, the only concentration tested.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** Laboratory reagent grade; 99% pure.  
13-DEC-1996 (1284)

**Type:** Somatic mutation assay  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** inhalation  
**Exposure period:** 22 hours per day/7 days per week/6 weeks  
**Doses:** 0, 40, 100, 1000 ppb

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Groups of 14 mice/sex were exposed to benzene in whole-body inhalation chambers. At sacrifice, the spleen of each mouse was removed and the lymphocytes released. The numbers of hypoxanthine-guanine phosphoribosyl transferase (hprt) mutant (variant) cells were determined according to an autoradiographic hprt mutant lymphocyte assay. Control groups of mice were either exposed to purified air or housed

in standard plastic cages.  
Reliability: 4 (validity could not be judged)  
Original reference not yet available

**Result:** Benzene caused a dose-dependent increase in the frequencies of hprt variant (mutant) lymphocytes in both male and female mice at concentrations of 40 and 100 ppb. At 1000 ppb, the frequency was also increase when compared with the controls but this was less than in the 40 and 100 ppb groups. Females were more sensitive than males.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1236)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male/female  
**Strain:** other: see method  
**Route of admin.:** oral feed  
**Exposure period:** up to 96 hours  
**Doses:** 0.88, 4.4, 8.8 mg/ml

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
one dose tested per experimental design  
The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. Benzene was administered to mei-9 and +/-mei-9 strain Drosophila as a solution in 5% Tween 80 and 5% ethanol. Larvae aged 48 or 72 hours were exposed for 2 hours to benzene concentrations of 4.4 and 0.88 mg/ml respectively. In some cases, larvae were treated during the entire larval life (96 hours) to 4.4 or 8.8 mg/ml. After exposure, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and the frequency and size of the spots were recorded. Control experiments were conducted by exposing larvae of the same age as those in the treatment groups to the 5% Tween 80 and 5% ethanol vehicle for the same length of time as the corresponding treated larvae.

**Result:** A benzene concentration of 8.8 mg/ml was lethal and 4.4 mg/ml toxic when given for the entire larval life. A significant increase in the frequency of small single spots and/or total single spots was seen in the acute (larvae aged 72 h) and chronic studies in the excision-repair-defective larvae (mei-9 strain). The acute result was only marginally positive. No such effect was seen when benzene was given to excision-repair-proficient larvae (+/mei-9 strain).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997 (1284)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** other  
**Route of admin.:** oral feed  
**Exposure period:** 96 hours  
**Doses:** 0, 0.5, 1, 2 % (converts to 0, 4.4, 8.8, 17.6 mg/ml)

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Benzene was dissolved in 40% ethanol. Male and female C(1)DX,yf/Y/sc z w+ mei-9a Drosophila were mated for 4-5 days and allowed to ovipost for 24 hours. The resultant eggs were allowed to develop for 24 hour and the newly hatched larvae exposed to benzene or the vehicle as the control. Benzene was added (1 ml) to the culture medium and the larvae were allowed to develop throughout the larval period (roughly 96 hours). Eyes of adult males were examined for colour mutations.  
 Reliability: 1 (valid)

**Result:** Test procedure in accordance with generally accepted scientific standards and described in sufficient detail  
 No significant increase in mutation frequency over the control levels was seen. Benzene, however, caused a dose-dependent toxic effect on the survival of the male larvae, with 50% survival occurring at the top dose. A similar study using the genotype sc z w+ and benzene concentrations of 8.8 and 17.6 mg/ml reported similar decreases in survival and a lack of mutagenic effect (Nylander P.-O. et al. Mutation Res. 57, 163-167, 1978).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
 06-JAN-1997

(380)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** female  
**Strain:** other  
**Route of admin.:** oral feed  
**Exposure period:** 10-11 days  
**Doses:** 156 mg/l

**Method:** other  
**Year:** 1957  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
 No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
 one dose  
 The method of Becker H.J. Z. Indukt. Abstammungs-Vererbungslehre 88, 333-373, 1957 was followed. Benzene was prepared in a mixture of 3% ethanol and 1% Tween 60 before being added to the food. Virgin females of the genotype wcosn2; se h were crossed with w; se h males and

allowed to lay eggs for a period of 4 days on the food supplemented with benzene. The control larvae were exposed to the solvent. The developing culture was incubated for 10-11 days and the hatching females were scored for genetic changes which produced w clones or wco//w twin spots in their eyes.

**Result:** No increase in the number of mosaic twin spots was observed. However, the numbers of mosaic single spots and twin and single spots were significantly increased. The authors regarded these as inconclusive as more data are needed for their final classification.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997 (1219)

**Type:** Somatic mutation assay  
**Species:** Drosophila melanogaster **Sex:** male/female  
**Strain:** other  
**Route of admin.:** inhalation  
**Exposure period:** 1 hour  
**Doses:** 0, 0.05, 0.2, 0.3 ml/1150ml (converts to 0, 38, 153, 229 mg/l)

**Method:** other  
**Year:** 1983  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
max. 2 doses tested per experimental design  
The method of Wurgler F.E. et al. Mutation Res. 122, 321-328, 1983 was followed. mwh+/+flr and mei+9 strain larvae were exposed at an age of 48 or 72 hours. They were placed in a 1150 ml airtight container and benzene was injected and allowed to volatilize to give the required concentration. After treatment, the wings of the surviving adults were mounted and scored for the presence of single and twin spots. The number of wings scored and frequency and size of the spots were recorded. Control groups were exposed to air at the same age and for the same length of time as the larvae in the treatment groups.

**Result:** An increase in the frequency of small single spots and/or total single spots was seen in excision-repair-proficient larvae (mwh+/+flr) exposed to 229 mg/l but not 153 mg/l and in excision-repair-defective larvae (mei-9) exposed to 38 mg/l, the only concentration tested.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; 99% pure.  
06-JAN-1997 (1284)

**Type:** other  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** other  
**Route of admin.:** inhalation  
**Exposure period:** 45 minutes  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** This test was conducted to examine the ability of benzene to induce crossing-over in Drosophila strain vg bw/++ males. Benzene (0.1 ml) was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. For the detection of induced crossing over, the five treated males were then individually back crossed to ten vg bw virgins for 18 days to obtain nine two-day broods. Broods were then scored for presence of crossovers. Laboratory cumulative control data were used as the comparison.

**Result:** Benzene induced crossing-over in spermatogonia but not in spermatozoa, spermatids or spermatocytes. The data were heavily clustered with more than half the crossovers coming from one individual treated male. The clusters were due to mitotic multiplication of crossover chromosomes in gonial cells and the investigators concluded that the observations could not be used for quantitative estimation of crossing-over induction. The crossovers were derived from four males and the difference when compared with the control group was found to be statistically significant. All four males found positive for induction of crossing-over in spermatogonia were sterile in the next brood.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996

(593)

**Type:** other  
**Species:** Drosophila melanogaster **Sex:** male  
**Strain:** other  
**Route of admin.:** inhalation  
**Exposure period:** 45 minutes  
**Doses:** 0, 27000 ppm (converts to 0, 88 mg/l)

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Reliability: 2 (valid with restriction)  
No standard test procedure, but in accordance with generally accepted scientific standards and described in sufficient detail  
no data about the TS  
This test was conducted to examine the ability of benzene to induce crossing-over in Drosophila strain vg bw/++ males. Benzene (0.1 ml) was injected onto a piece of filter paper in the exposure chamber and allowed to volatilize to produce

the required concentration. Males were transferred to fresh food immediately after treatment and maintained for 2 days. For the detection of induced crossing over, the five treated males were then individually back crossed to ten vg bw virgins for 18 days to obtain nine two-day broods. Broods were then scored for presence of crossovers. Laboratory cumulative control data were used as the comparison.

**Result:** Benzene induced crossing-over in spermatogonia but not in spermatozoa, spermatids or spermatocytes. The data were heavily clustered with more than half the crossovers coming from one individual treated male. The clusters were due to mitotic multiplication of crossover chromosomes in gonial cells and the investigators concluded that the observations could not be used for quantitative estimation of crossing-over induction. The crossovers were derived from four males and the difference when compared with the control group was found to be statistically significant. All four males found positive for induction of crossing-over in spermatogonia were sterile in the next brood.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(593)

**Type:** other: DNA adducts  
**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of admin.:** gavage  
**Exposure period:** 10 weeks  
**Doses:** 0, 200, 500 mg/kg  
**Method:** other: nonguideline  
**Year:** 1989  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** A nuclease P1-enhanced <sup>32</sup>P-postlabeling assay, having a sensitivity limit of 1 adduct in 10(9-10) DNA nucleotides, was found suitable for measuring aromatic DNA adducts derived in vitro from catechol, benzenetriol (BT), phenol, hydroquinone (HQ), and benzoquinone (BQ), potential metabolites of benzene. When DNA specimens isolated from tissue of female Sprague-Dawley rats at 24 hr after an oral gavage dose of 200 to 500 mg/kg, 5 days/week, in olive oil (3 mL/Kg) for 1 day, 1 week, 5 weeks, and 10 weeks were analyzed by the <sup>32</sup>P-postlabeling procedure, no aromatic adducts were detected unequivocally with DNA samples of liver, kidney, bone marrow, and mammary gland. With Zymbal gland DNA, three weak spots at levels totaling four lesions per 10(9) DNA nucleotides were seen only after 10 weeks of treatment, and these adducts did not correspond chromatographically to major adducts in vitro from the abovespecified compounds. This distinct adduct pattern may relate to tumor induction in this organ following benzene administration. The results also indicate that DNA adducts derived from catechol, BT, phenol, HQ, and BQ are either not formed in vivo with benzene or formed at levels below the detection limit of 1 adduct per 10(9-10) DNA nucleotides.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(930)

**Type:** other: Micronucleus Assay and Metaphase Analysis  
**Species:** mouse **Sex:** male/female  
**Strain:** CD-1  
**Route of admin.:** gavage  
**Exposure period:** 30 to 54 hours  
**Doses:** 440 mg/kg

**Method:** other: Gad-El-Karmim et al.  
**Year:** 1984  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** The effects of benzene on bone marrow was studied in the micronucleus assay and by metaphase analysis. Male and female CD-1 mice were treated with 2 doses of benzene (440 mg/kg) or toluene (860 or 1720 mg/kg) or both 24 hours apart, and sacrificed 30 hours (or 54 hours) after the firstdose.

Benzene treated animals were pretreated with phenobarbital, 3-methylcholanthrene, SKF-525A or Aroclor 1254 by i.p. injection. Toluene showed no clastogenic activity and reduced the clastogenic effect of benzene when the mixture was given. None of the pretreatments protected against the clastogenic effects of benzene. However, pretreatment with 3-methylcholanthrene greatly promoted the clastogenic effects of benzene on bone marrow. Females were consistently less sensitive to benzene than males. Dose-response curves in benzene-treated mice were much steeper with 3-MCA induction than without. Chromosomal damage was higher with p.o. than i.p. administration of benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(387)

**Type:** other: Retrospective Analysis of Micronucleus Formation from the NTP Benzene Bioassay  
**Species:** mouse **Sex:** male/female  
**Strain:** B6C3F1  
**Route of admin.:** gavage  
**Exposure period:** 120 days to 2 years

**GLP:** yes  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Retrospective analysis of peripheral blood obtained during the NTP Cancer Bioassay of Benzene demonstrates that the frequency of micronuclei in circulating normochromatic erythrocytes (NCE's) reflects the clastogenic effect of benzene and sex sensitivity in mice. Peripheral blood was evaluated from mice subject to the following exposure schemes. In subchronic studies, benzene was administered by gavage to mice (5/group), at doses of 0, 25, 50, 100, 200, 400 or 600 mg/kg benzene in corn oil, 5 days/week for a 120 day period. In chronic studies, mice (50/group) were

exposed at 0, 25, 50 or 100 mg/kg benzene in corn oil for 24 months. An additional 10 mice/group were sacrificed at 12 months. In all cases, a total of 1000 NCE's were scored per animal.

Study results demonstrated a statistically significant dose dependent increase in micronucleated NCE's at all sampling times. The frequency of micronucleated NCE's was higher in male than in female mice. In treated males, the frequency was highest in the 120-day samples. In females, the responses to long term exposure were too weak to permit comparison around time points. Evaluation of the control animals from 120 day study found no statistical increase in the frequency of micronucleated polychromatic erythrocytes (PCE's) versus NCE's. This finding suggests that micronucleated NCE's are not selectively removed from peripheral circulation in B6C3F1 mice.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (200)

**Type:** other: Mammalian sperm-abnormality assay  
**Species:** mouse **Sex:** male  
**Strain:** other: see remarks  
**Route of admin.:** i.p.  
**Exposure period:** once daily for 5 days  
**Doses:** 0, 0.1, 0.25, 0.4, 0.5, 0.6, 0.8, 1.0 ml/kg bw/day (converts to 0, 88, 220, 352, 440, 527, 703, 880 mg/kg bw/day)

**Method:** other  
**Year:** 1980  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Reliability: 1 (valid)  
Test procedure in accordance with generally accepted scientific standards and described in sufficient detail. The method of Topham J.C. Mutation Res. 69, 149-155, 1980 was followed. The animals tested were the F1 generation derived from mating CBA male mice with BALB/c females. Groups of five were exposed to each dose. Five weeks after the final dose, smears of caudal sperm were examined for head abnormalities. Cyclophosphamide monohydrate was used as the positive control. Benzene was administered in corn oil and negative control animals were treated with corn oil alone.

**Result:** Benzene administration caused a significant increase in sperm-head abnormalities. Although this occurred over a limited dose range, it was reproducible in repeat experiments.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** Laboratory reagent grade; purity not specified.  
06-JAN-1997 (1146)

**Type:** other: activation of p53 in bone marrow  
**Species:** mouse **Sex:**

**Method:** ANIMALS AND TREATMENTS  
C57BL/6 (wild-type p53 +/+) and C57BL6 p53 +/- (N%) mice (8-9 wk old) were exposed whole body to benzene vapor (10 hr/d on Mon, Wed, Fri) for 15 wk. The concentration of benzene within the chamber (100 +/- 0.7 ppm) was monitored every 30 min (IR). The concentration of benzene at 9 points within the chamber varied by less than 8%. Controls were exposed to room air while inside another exposure chamber.

ISOLATION OF BONE MARROW  
The mice were euthanized, and femoral bone marrow removed by flushing with "RNA Later" solution (Ambion, Austin, Tx) and stored at 4 degrees. Total RNA was quantified spectrophotometrically using a proprietary kit.

QUANTITATIVE RT-PCR  
Prior to real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR), genomic DNA was removed by treatment of the sample with DNase-1. RNA from individual marrow samples was then reverse transcribed up to 3 times using 9 forward/reverse primers (designed using "Primer Express" software). Quantitative RT-PCR was performed in triplicate using "SYBR Green" (Perkin Elmer) with the ABI PRISM 7700 Sequence Detection System (Perkin Elmer) and GAPDH as the calibrator gene. All expression levels for a particular gene were expressed relative to the expression levels present in the air-exposed controls: values of less than one indicate that benzene exposure resulted in decreased expression for that particular gene, while values greater than one were indicative of an induction of gene expression.

CELL CYCLE ANALYSIS  
Bone marrow cells were obtained from the humeri of control and exposed mice of both genotypes. Cells were washed twice with 70% ethanol, stained with propidium iodide prior to analysis with flow cytometry.

STATISTICAL METHODS  
Data were analysed using Student's T-test (one-tailed).

**Remark:** C57BL/6 Trp53 heterozygous (N5) mice exhibit an increased sensitivity to tumorigenesis following exposure to genotoxic compounds. The authors hypothesised that heterozygosity at the p53 locus in these animals altered the expression of bone marrow p53-regulated genes involved in cell cycle control and apoptosis during chronic exposure to genotoxins. Human cancers progress from a homozygous wild type p53 state, though a heterozygous state, eventually resulting in a complete loss of p53 function.

**Result:** GENES INVOLVED IN P53 REGULATION  
Significantly greater amounts of p53 mRNA were present in bone marrow cells from benzene-exposed p53+/+ mice compared to benzene-exposed p53+/1 mice. Hyterozygosity of p53 also

resulted in significantly higher levels of mdm-2 and p19 in bone marrow cells after benzene exposure.

#### GENES INVOLVED IN CELL CYCLE CONTROL

p21 mRNA was increased 17-fold in bone marrow from p53+/+ mice following exposure to benzene, while a more modest (5-fold) increase in p53+/- cells. Expression of gadd45 doubled in p53+/+ cells after benzene exposure (unchanged in p53+/-) while levels of benzene-induced cyclin G were increased 3-5 fold in both genotypes (ie no effect of p53 heterozygosity).

#### EXPRESSION OF APOPTOSIS

Levels of bax in bone marrow cells from benzene-exposed p53+/+ mice were approx. double those in exposed p53+/- mice, while bcl-2 was slightly lower in homozygous mice compared to heterozygous mice.

#### CELL CYCLE ANALYSIS

Bone marrow from p53+/+ mice exposed to benzene showed a significant increase in the G0/G1 fraction as well as a significant decrease in the S fraction of cells. In contrast, the G2/M fraction was increased, and the S fraction decreased, in cells from p53+/- mice.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

Heterozygosity at the p53 locus results in a haplo-insufficient phenotype expressing an altered expression of key genes which regulate the DNA damage response pathway. This compromised response may be a central factor in the increased susceptibility of p53+/- mice to accelerated tumorigenesis following exposure to genotoxic carcinogens.

04-MAR-2003

(131)

**Type:**

other: aneuploidy in mice

**Method:**

Animals and treatments

Male and female C57B1/6J mice (8 - 12 wk old, n = 2 or 3 per group) were given 220, 440 or 880 mg/kg bwt by gavage in corn oil (200ul/mouse). Treatments were either a single dose or repeated daily for 2 - 4 days.

#### Sample collection and processing

Peripheral blood was collected by heart puncture and bone marrow from femur and tibia (all sampled 6 d, 9 wk or 8 months post-treatment). Marrow sub-populations containing immature/primitive cells were discriminated and isolated using flow cytometry. Viable cells (with the phenotype Lin-minus c-kit+ Sca-1+) were isolated and incubated with cytokines to promote expansion into myeloid/erythroid or lymphoid lineages.

#### Aneusomy quantification

The frequency of cells with aneusomies was quantified using FISH with DNA probes for mouse chromosomes 2 and 11. Where aneusomy was present a minimum count of at least 37

chromosomes was performed per sample.

#### Haematopoietic cytotoxicity

The frequency of myeloid progenitor cells (colony forming units-granulocytic macrophage; CFU-GM) was quantified using a cytokine-enriched proprietary assay (Methocult <sup>TM</sup>).

#### Statistics

Student's t-test was used to compare CFU-GM frequencies. Aneusomy frequencies were compared using chi-square test, with Cochran's test to identify any dose-response relationship present.

#### Result:

##### Effects on haematopoiesis

Blood and marrow cellularity of control and benzene-treated mice were similar at all measurement intervals (i.e 6 d, 9 wk and 8 mo post-treatment). The frequency of primitive/immature cells with the Lin-minus c-kit+ Sca-1+ phenotype and marrow CFU-GM was similar in control and benzene-treated mice, indicating minimal direct toxicity on immature haematopoietic cells.

##### Aneusomy in haematopoietic sub-populations

The frequency of circulating lymphocytes and marrow myeloid and hsc-containing sub-populations with copy changes in chromosomes 2 and 11 were quantified 6 d, 9 wk and 8 mo post-treatment. In benzene-treated mice, the frequency of aneuploid lymphocytes and myeloid cells was higher at 9 wk than at 6 d, suggesting disruption of chromosomal segregation in differentiating cells and/or progenitors. About 8 mo after benzene treatment, approx. 14% of cells with the Lin-minus c-kit+ Sca-1+ phenotype exhibited numerical chromosomal aberrations affecting chromosomes 2 or 11.

#### Source:

A.K. Mallett Surrey

#### Conclusion:

The results indicate that large oral doses of benzene induce copy changes in chromosomes present in immature/primitive cells, and that these changes persist for several months. However the contribution of benzene-induced aneuploidy in immature/primitive cells to leukemogenesis remains to be determined.

25-APR-2002

(413)

#### Type:

other: aneuploidy in oocytes

#### Species:

mouse

#### Sex:

#### Remark:

Chinese language article, translation unavailable:

#### ABSTRACT:

"Mice were treated with various doses of benzene (942, 1,881 and 3,762 mg/kg respectively) via single gavage and multiple inhalation (706, 1,922 and 4,864 mg /m<sup>3</sup>, respectively). After gavage, the mice were pair-matched (1:1) with males overnight. The ovulated oocytes and the one cell zygotes were collected for cytogenetic analysis and the frequencies of aneuploidy were detected.

The frequencies of aneuploidy in M II oocytes significantly

increased in the three groups treated by inhalation (7.06%, 7.50% and 7.76%, respectively, control group 0.00%, P 0.05) of benzene, as compared with those in the control group (1.30%), with a dose-dependent response. Meiotic delay of M I oocytes was observed, with the frequency of M I oocytes of 1.16%, 3.61% and 5.75%, respectively, P 0.05, in a dose-dependent response, too. In gavage treatment group, increase in frequency of M II oocyte aneuploidy was only observed in the high-dose group, and aneuploidy frequency of female pronucleus in one cell zygotes was not induced."

**Source:** A.K. Mallett Surrey  
**Conclusion:** Exposure to high-doses of benzene via gavage or inhalation increased the frequency of aneuploidy in mouse M II oocytes.  
04-MAR-2003 (1307)

**Type:** other: chromosomal aberrations in benzene-exposed humans  
**Species:** human **Sex:**

**Method:** SUBJECTS AND METHODS  
One hundred and thirty subjects with occupational exposure to benzene were recruited from factories in the Tianjin region of China. Fifty-one control subjects were recruited from nearby food processing factories. Inclusion criteria included at least 3 years employment with no known disease (as assessed by interview and tests of liver function and urine analysis). The study was conducted in 2 phases in spring 1997 (25 exposed + 25 unexposed) and spring 1998 (26 unexposed + 105 exposed).

#### EXPOSURE ESTIMATES

Current personal benzene exposure was assessed on 3-4 occasions over a one month period using passive monitoring (3M organic vapour monitors). A 4 week mean exposure level and lifetime cumulative exposure (based on historic area sampling records and the subject's work history) were also calculated.

#### CHROMOSOMAL ABERRATION ASSAYS

A sample of peripheral venous blood (heparinised) was collected after a workshift and stored at 4 degrees C prior to transport to the USA for processing. One hundred well-spread metaphase cells containing at least 45 chromosomes were scored per subject for structural aberration using a brightfield microscope (x 100 magnification). Numerical aberrations were scored by recording the number of metaphase spreads containing 44 or fewer chromosomes, 45 chromosomes, 47 or more chromosomes or polyploidy.

#### STATISTICAL METHODS

Exposure-related trends were identified using ANOVA linear contrast.

**Result:** CHROMOSOMAL ABERRATIONS  
There was a significant increase in aberrations when results were analysed using 4 wk mean exposures, due primarily to an

increase in chromatid breaks (although other aberrations such as chromosomal breaks appeared to contribute to the trend). A modest increase in mitotic index (metaphases per 1000 cells) was also seen with increasing benzene exposure, due almost entirely to an increase in metaphases in samples from workers with the highest exposure. When adjustments were made for possible confounding factors, and benzene exposure was treated as a continuous variable, there were moderate associations of benzene exposure with chromosomal gaps and chromosomal breaks only.

Examination of responses in a sub-set of workers where the 4 wk mean benzene exposure was 0.5 ppm or lower revealed positive associations for total chromatid aberrations (excl. gaps), total chromosome aberrations (excl. gaps), total aberrations (excl. gaps), chromatid breaks and acentric fragments:

	Unexposed	Exposed
No. subjects	51	16
Female (%)	53	100
Smoker (%)	31	0
Age (yr)	33	36
4 wk mean benzene (ppm)	0.004	0.14
Total chromatid abs. (%)	1.2	2.2 (P = 0.01)
Total chromosome abs. (%)	0.6	1.4 (P = 0.008)
Total aberrations (%)	1.8	3.6 (P = 0.001)
Chromatid breaks (%)	1.2	2.2 (P = 0.01)
Acentric fragments (%)	0.4	1.1 (P = 0.01)

Comment: The authors note that aberration data obtained for individuals exposed to 0.5 ppm or lower were comparable to values for subjects exposed to 30 ppm and above. Further analysis indicated a longer median exposure duration for the low exposure group (14 yr) compared to the high exposure group (3.25 yr). Hence changes in the low exposure workers may have been due to past cumulative exposure to benzene.

Analysis by lifetime cumulative benzene exposure showed significant associations for total chromatid aberrations, total chromosomal aberrations, total aberrations, chromatid breaks and acentric fragments.

When the association of chromosomal aberrations were analysed with respect to cumulative benzene exposure, exposure duration (i.e. no. years of exposure; median = 14 yr) was predictive of total chromatid aberrations, total aberrations, chromatid breaks and acentric fragments.

Exposure intensity (i.e. estimated mean exposure concentration per yr; median = 2.7 ppm/yr) was found to be predictive of chromatid gaps and chromosomal breaks.

#### ANEUPLOIDY

Only hypodiploidy (45 chromosomes) showed a positive association relative to 4 wk mean benzene exposure levels.

There was no change in the type of aneuploidy when unexposed workers were compared with workers exposed to a 4 wk mean exposure of 0.5 ppm or lower.

Lifetime cumulative benzene exposure and benzene exposure intensity (but not exposure duration) were significantly associated with changes in chromosome number.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

Chromosomal aberration data showed significant increases in chromatid breaks and total chromosomal aberrations in exposed subjects compared with unexposed subjects. While aberration frequencies were increased in subjects with mean benzene exposures of 0.5 ppm or lower, it is unclear if this reflected chromosomal damage associated with current or past exposure. Overall, duration of exposure appeared more critical and predictive of the presence of chromosomal aberrations than current benzene exposures.

16-MAR-2004

(915)

**Type:**

other: chromosomal aberrations in human populations

**Remark:**

This review provides a detailed summary and analysis of investigations of chromosomal aberrations (CA) in benzene-exposed workers, benzene "poisoned" subjects and preleukemia patients, and leukemia cases associated with benzene exposure. It includes the following elements:

- \* Historical background
- \* Chromosomal aberrations as a biomarker for cancer risk
- \* Chromosomal aberrations reported in benzene-associated leukemia cases
- \* Chromosomal aberrations detected in preleukemia and benzene-poisoned patients
- \* Cytogenetic changes detected in benzene-exposed (non-diseased) individuals
- \* Application of FISH in benzene-exposed workers
- \* Comparison of benzene to other leukemogens
- \* Conclusion and future directions

The authors note that CA have been detected in many human studies, mostly as gaps and breaks. Aneuploidy, including hyperploidy and hypoploidy, has been observed frequently. The loss and gain of chromosomes in the C-group appear especially common among benzene-associated leukemia and preleukemia patients. There is evidence that chromosome-type aberrations (involving both chromatids) may persist some time (years) after recovery from benzene-induced haematotoxicity. Application of molecular cytogenetics, in particular fluorescent in situ hybridisation (FISH), has aided in the rapid analysis of specific CA among benzene-exposed populations. Increased levels of aneuploidy and structural changes associated with leukemogenesis have been detected using FISH in many studies involving highly exposed workers. They suggest that benzene may act both as an alkylating agent causing alterations in chromosomes 5 and 7, and a topoisomerase II inhibitor inducing t(21q22).

**Source:** A.K. Mallett Surrey (1309)  
26-JAN-2003

**Type:** other: human SCE and CA

**Method:** SUBJECTS  
Blood samples (peripheral venous blood) were collected from 25 healthy control subjects (5 men, 20 women; average age 38 yr; 20% smokers) and 9 donors with occupational exposure to benzene (8 men, 1 women; average age 43 yr; 22% smokers; no further information on benzene exposure provided). (Samples were also collected from small groups of individuals (n=10-13) with putative exposure to styrene, polycyclic aromatic hydrocarbons and 'mixed solvents'.)

DETERMINATION OF CHROMOSOME ABERRATION (CA)- AND SISTER CHROMATID EXCHANGE (SCE) FREQUENCIES

Samples of heparinised blood were incubated in RPMI-1640 foetal calf serum with phytohaemagglutinin-P for 50 hr (CA) or 72 hr (SCE). Cell culture, harvest and slide preparation followed standard methods. Bromodeoxyuridine (BrdU) was used in the SCE analyses to identify the first and subsequent metaphases. Slides for evaluation of CA were stained using Giemsa, while those for SCE counts used the "Fluorescent-Plus-Giemsa" method. Microscopic analyses were performed 'blind'. SCE frequencies were expressed as the mean number of exchanges per mitosis. CA were characterised in 100 metaphases with 46 +/-1 chromosome per subject, and expressed as the percentage aberrant cells. (Achromatic lesions (gaps) and/or aneuploidy were not considered aberrant, while a CA frequency of >4 was considered positive.)

STATISTICAL METHODS

Statistical differences between control and exposed subjects were analysed using Student's T-test.

**Result:** Mean CA- and SCE frequencies were elevated (by 105% and 9%, respectively) in the benzene-exposed group, however neither increase was statistically significant. (Comment: SCE and CA were also numerically greater in the other populations, however statistical significance was achieved only in the styrene (SCE), "mixed solvent" (SCE) and PAH (CA, SCE) groups.)

**Source:** A.K. Mallett Surrey

**Conclusion:** No significant effect on chromosomal aberrations or sister chromosome exchanges was found in this study.

04-MAR-2003 (107)

### 5.7 Carcinogenicity

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** life-time  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** none  
**Doses:** 300 ppm (converts to 0.98 mg/l); 40 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** C57Bl/6J strain mice were used. Control group consisted of 40 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every ½-hour during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 488 days when the last test animal died.

**Result:** Median survival time and body weight gain were decreased in treated mice. Haematological studies were suspended after 61 weeks as too few animals were alive in the test group. Statistically significant increases in lymphocytopenia and anaemia were seen throughout the study and neutrophilia was evident after 17 weeks. Changes were observed in the peripheral blood cell morphology of the treated animals including anisocytosis (variation in red blood cell size), poikilocytosis (variation in red blood cell shape), giant platelets and, concurrent with neutrophilia, hyperlobulated, mature neutrophils and neutrophilic left shift. On histopathologic examination, the incidences of haematopoietic neoplasms and bone marrow and splenic hyperplasias (without evidence of haematopoietic neoplasm) were statistically significantly increased when compared with the control group (8/40, 13/32 and 10/32 in the treated and 2/40, 0/38 and 2/38 in the control group for the three tumours respectively). Six of the eight test animals showing haematopoietic neoplasms had lymphocytic lymphoma with thymic involvement, a significant increase when compared with the two control animals showing lymphocytic lymphoma without thymic involvement.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

01-JUL-2005

(1066)

**Species:** mouse **Sex:** male  
**Strain:** other:AKR/J  
**Route of administration:** inhalation  
**Exposure period:** life-time  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** none  
**Doses:** 100 ppm (converts to 0.33 mg/l); 50 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** AKR/J strain mice were used. Control group consisted of 50 males exposed to filtered, conditioned air under the same exposure regime as above. Benzene concentrations in the exposure chambers were determined every ½-hour during the daily exposures. Animals were observed daily for evidence of morbidity and weighed weekly for the first 4 weeks and biweekly thereafter. Blood was withdrawn from ten test and ten control mice every other week throughout the study. The lung, liver, spleen, kidney, bone marrow and any abnormally appearing organs were examined histologically. Exposure ended after 505 days when the last test animal died.

**Result:** No statistically significant difference in median survival time or on the rate of weight gain was noted. Haematological studies were suspended after week 37 as too few animals remained due to advancing mortality. Statistically significant lymphocytopenia was observed throughout the study, from week 1. Red blood cell levels were consistently depressed (anemia) and there was some tendency toward neutrophilia throughout the study. Little or no morphological differences were seen in the peripheral blood. Malignant lymphoma occurred in 29 of the 49 treated and 24 of the 50 control animals. The predominant type in both groups was disseminated, lymphocytic lymphoma with thymic involvement. Of the 29 treated mice with lymphoma, 15 had lymphoma cell infiltrates in the bone marrow, 7 had bone marrow hypoplasia and 7 had essentially normocellular bone marrows. The corresponding figures in the 24 control animals were 5, 1 and 18 respectively. In the treated group as a whole, ten of the animals (20%) had bone marrow hypoplasia in comparison with one of the control mice, a statistically significant increase.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

01-JUL-2005

(1066)

**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of administration:** inhalation  
**Exposure period:** life-time  
**Frequency of treatment:** 6 hours per day/5 days per week/1 week interrupted by 2 weeks  
**Post exposure period:** 2 weeks observation after each week of exposure  
**Doses:** 300 ppm (converts to 0.98 mg/l); 60 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 60 males exposed to filtered conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 129.

**Result:** No increased incidences of leukaemia /lymphomas. However, male CD-1 mice repeatedly exposed to 300 ppm (960 mg/m<sup>3</sup>) benzene on 5 days (6 hr/d) interrupted by 2 weeks of non-exposure until death exhibited increased incidences of tumors (total numbers), malignant tumors and lung tumors in CD-1 mice.

The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study. When the tumour types were grouped together, all the incidences were increased; that is, the numbers of total, malignant and benign tumour-bearing animals, lung adenomas, leukaemias or lymphomas (not significant) and Zymbal gland (not significant) carcinomas (25, 12, 15, 14, 7 and 2 of the 54 treated mice and 4, 1, 3, 3, 1 and 0 of the 46 control animals for each tumour class respectively). The incidences of tumours in the individual tissue types were not recorded.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** Chromatoquality grade benzene was tested.

**Flag:** Risk Assessment

01-JUL-2005

(1065)

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** life-time  
**Frequency of treatment:** 6 hours per day/5 days per week/1 week in every 3  
**Post exposure period:** 2 weeks observation after every week of exposure  
**Doses:** 300 ppm (converts to 0.98 mg/l); 60 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** C57Bl/6J strain mice were used. Control group consisted of 60 males exposed to filtered, conditioned air in chambers, using the same intermittent exposure regime. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood samples were taken from ten exposed and ten control animals at the end of each week of exposure. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically. Total number of days exposed was 181.

**Result:** The mortality rate was increased by benzene exposure and lymphocytopenia and anaemia were seen throughout the study.

No increased incidences of leukaemia /lymphomas were observed. However, male C57Bl/6J mice repeatedly exposed to 300 ppm (960 mg/m<sup>3</sup>) benzene on 5 days (6 hr/d) interrupted by 2 weeks of non-exposure until death exhibited increased incidences of tumors (total numbers 25/54 vs. 8/46 controls), malignant tumors (14/54 vs. 2/46 controls) and zymbal gland tumors (19/54 vs. 0 /46 controls).

No increase in the numbers of benign tumour-bearing animals or lung adenomas were recorded. The incidences of tumours in the individual tissue types were not recorded.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** Chromatoquality grade benzene was tested.

**Flag:** Risk Assessment

01-JUL-2005

(1065)

**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of administration:** inhalation  
**Exposure period:** 10 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** until spontaneous death  
**Doses:** 1200 ppm (converts to 3.92 mg/l); 80 mice; mean analytical daily concentration 1195 +/- 41 ppm  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at half-hour intervals during the daily exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.

**Result:** The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, statistically significant increases were seen in the incidences of total, malignant and benign tumour-bearing animals, lung adenomas and Zymbal gland carcinomas when compared with the untreated controls (numbers = 45, 24, 35, 33 and 4 of the 71 treated mice compared with 36, 22, 21, 17 and 0 in the control group of 71 mice, for each tumour class respectively). No increase in the numbers of leukaemias or lymphomas were reported. The incidences of tumours in the individual tissue types were not reported.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** Chromatoquality grade benzene was tested.  
**Flag:** Risk Assessment

01-JUL-2005

(1064)

**Species:** mouse **Sex:** male  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** 10 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** until spontaneous death  
**Doses:** 1200 ppm (converts to 3.92 mg/l); 80 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** C57Bl/6J strain mice were used. Control group consisted of 80 males exposed to filtered, conditioned air in chambers, 6 hours/day, 5 days/week for 10 weeks and observed until spontaneous death. Benzene concentrations in the exposure chambers were monitored at ½-hour intervals during the daily exposures. Blood was withdrawn from ten exposed and ten control animals every second week during the exposure period and monthly thereafter. At the end of the study, autopsy was performed on all the animals and the lung, liver, spleen, kidney, bone marrow, thymus, head, lymph nodes and any abnormally appearing organs examined histologically.

**Result:** The mortality rate was increased by benzene exposure and intense lymphocytopenia and mild anaemia were noted during the exposure period. The blood counts returned to normal when exposure ceased. When the tumour types were grouped together, an increase in the numbers of Zymbal gland carcinomas were found (4 of the 68 treated mice compared with 0 in the control group of 67 mice). No increases in total, malignant or benign tumour-bearing animals, lung adenomas and leukaemias or lymphomas were noted. The incidences of tumours in the individual tissue types were not recorded.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** Chromatoquality grade benzene was tested.  
**Flag:** Risk Assessment

01-JUL-2005

(1064)

**Species:** mouse **Sex:** female  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** 16 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** until spontaneous death  
**Doses:** 300 ppm (converts to 0.98 mg/l); 118 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** C57Bl/6 strain mice were used. Control group consisted of 116 females exposed to air. On days 3, 28, 56, 112, 168 and 179 after the last exposure, five mice (three on day 179) from each group were killed for haematopoietic stem cell assays. Necropsy was performed on all animals and gross and microscopic appearance of the thymus, lungs, spleen, liver, kidneys and bone marrow evaluated.

**Result:** see also Cronkite et al., 1985  
An increase in mortality was seen 48 weeks after the end of the exposure period in the benzene-treated animals. Of the ten animals which died, six were found to have thymic lymphoma and two, unspecified lymphomas. Of the remaining two deaths, one mouse was killed in a moribund condition and found to be leukaemia free while the other was undiagnosed due to autolysis and partial cannibalism. This increase in leukaemia was statistically significantly greater than the incidence seen in the control group (one control mouse was killed moribund and had no histologic evidence of lymphoma-leukaemia) or in a group of 354 recent historical controls of this same sex and strain from the same laboratory.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Test substance:** Reagent grade, thiophene-free benzene was tested.

**Flag:** Risk Assessment

01-JUL-2005

(257)

**Species:** mouse **Sex:** female  
**Strain:** C57BL  
**Route of administration:** inhalation  
**Exposure period:** 16 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** 16 weeks  
**Doses:** 300 ppm (converts to 0.98 mg/l); 89 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** C57Bl/6 strain mice were used. Control group consisted of 88 females exposed to conditioned air. At various times during and after the exposure period, five to ten mice were removed from both the treated and control groups and the blood, bone marrow and spleens removed and examined.

**Result:** Early mortality and an increase in mortality rate were noted in the benzene-treated group. All leukaemias and, in particular, thymic and nonthymic lymphocytic neoplasms, benign and malignant Zymbal gland tumours and ovarian tumours were increased in the treated animals when compared with the control animals (incidence = 22.5, 11.2, 6.7, 18.0, 9.0 and 4.5% in the treated and 9.1, 1.1, 2.3, 1.1, 0 and 2.3% in the controls for the tumour types respectively).

The total number of lymphoma/leukaemia was 20/89 in exposed females (10 thymic neoplasias (lymphocytic), 6 nonthymic neoplasias (lymphocytic), 4 leukaemia not otherwise specified (NOS)) versus 8/88 in the control group (1 thymic neoplasia (lymphocytic), 2 nonthymic neoplasias (lymphocytic), 3 myelogenous leukaemia, 2 leukaemia NOS).

**Source:** see also Cronkite et al., 1984  
BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

01-JUL-2005

(256)

**Species:** mouse **Sex:** male  
**Strain:** CBA  
**Route of administration:** inhalation  
**Exposure period:** 16 weeks  
**Frequency of treatment:** 6 hr/day, 5 days/week  
**Post exposure period:** > 2 years  
**Doses:** 10, 25, 100, 300, 400 and 3000 ppm  
**Control Group:** yes, concurrent no treatment

**Method:** other: no guideline  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Two weeks of inhaling 10 ppm produced no hematologic effects; 25 ppm induced a significant lymphopenia. Inhalation of 100, 300, and 400 ppm produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, marrow content of spleen colony-forming units (CFU-S) and an increased fraction of CFU-S in DNA synthesis. Exposure of mice to 300 ppm for 2, 4, 8, and 16 weeks produced severe lymphopenia and decrease in marrow CFU-S. Recovery was rapid and complete after 2 and 4 weeks of exposure. After 8 and 16 weeks of exposure, recovery of lymphocytes was complete within 8 weeks. It took 16 weeks for the CFU-S to recover to that of the age-matched controls after 8 weeks of exposure and 25 weeks to recover to age-matched after 16 weeks of exposure. Inhalation of 3000 ppm for 8 days was less damaging than inhalation of 300 ppm for 80 days (same integral amount of benzene inhaled).

Exposure of CBA/Ca BNL mice to 100 or 300 ppm benzene for 16 weeks (6 hr/d, 5 d/w) resulted in myelogenous neoplasias in 19% of the male mice and 11% of the female mice at the dose of 300 ppm (960 mg/m<sup>3</sup>) versus 0% and 1.7% in control males and females. At 100 ppm (320 mg/m<sup>3</sup>), myelogenous neoplasms were observed in 2.4% of exposed males, but no case occurred in control males.

No incidence data for the other test concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Flag:** Risk Assessment

01-JUL-2005

(260)

**Species:** mouse **Sex:** male  
**Strain:** CD-1  
**Route of administration:** inhalation  
**Exposure period:** life-time  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** none  
**Doses:** 300 ppm (converts to 0.98 mg/l); 40 mice  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 40 mice exposed to air. Semi-monthly haematologic studies were conducted on ten test and ten control animals. Reliability: 3 (not valid) Significant methodological deficiencies one sex, no data about measured benzene concentration and test substance, insufficient documentation of methods, low number of animals, one concentration tested, results limited to leukaemia

**Result:** Three mice showed myeloproliferative disorders, including one case of acute myelogenous leukaemia, one case of chronic myelogenous leukaemia and a third in which granulocytic hyperplasia was present. No such effects were seen in the control group. Although the increased incidences are not statistically significant when compared with the control group, the fact that myelogenous leukaemia has not been reported in historic control data on this strain of mouse is suggestive of a causative role for benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

01-JUL-2005

(424)

**Species:** mouse **Sex:** male  
**Strain:** CBA  
**Route of administration:** inhalation  
**Exposure period:** 16 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Post exposure period:** 18 months  
**Doses:** 300 ppm (converts to 0.98 mg/l); 125 mice; mean analytical chamber concentration 300+/-3 ppm  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** CBA/Ca strain mice were used. Control group consisted of 125 animals exposed to conditioned, filtered air. Benzene concentrations in the exposure chambers were monitored twice per hour during the exposures. The experiment was terminated when the survival of the benzene-exposed group reached 20% (22 months from the start of exposure). Blood and bone marrow smears were examined from the 24

benzene-exposed mice alive at the terminal kill and 24 randomly selected controls. The major organs were examined histologically.

**Result:** A statistically significant increase in the mortality rate was seen in the benzene-treated group. Benzene-induced malignant lymphoma with metastasis was a significant cause of early mortality with 11 deaths due to lymphoma occurring in the first 9 months postexposure. For the total exposure period, the incidence of malignant lymphoma was significantly greater in the treated (14 cases/118) than the control (2 cases/119) group. Significantly increased incidences of preputial gland squamous cell carcinomas (60% in treated, 0% in control group), lung adenomas (36 and 14% respectively) and not significantly increased Zymbal gland carcinomas (11 and 1% respectively), forestomach squamous cell carcinomas (7 and 0% respectively) and granulocytic hyperplasias of the bone marrow (36 and 8% respectively) and the spleen (6 and 0% respectively) were found. Of the 24 treated and control mice alive at the terminal kill, no cytologic lesions were found in the bone marrow smears. Minimal to marked granulocytic hyperplasia was present in the bone marrow of 14 of the exposed animals and minimal granulocytic hyperplasia in two of the controls.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** 100% pure (analysed)

**Flag:** Risk Assessment

01-JUL-2005 (346)

**Species:** rat **Sex:** male

**Strain:** Sprague-Dawley

**Route of administration:** inhalation

**Exposure period:** life-time

**Frequency of treatment:** 6 hours per day/5 days per week

**Post exposure period:** none

**Doses:** 100, 300 ppm (converts to 0.33, 0.98 mg/l); 40 and 45 rats respectively

**Control Group:** yes, concurrent vehicle

**Method:** other

**GLP:** no data

**Test substance:** no data

**Remark:** Control group consisted of 40 and 27 rats for the two exposure groups respectively, exposed to air. Semi-monthly haematologic studies were conducted on ten test and ten control animals.

**Result:** One animal exposed to 0.33 mg/l developed chronic myelogenous leukaemia. Although not statistically significant when compared with the control group, where no such leukaemias were seen, the fact that myelogenous leukaemia has not been reported in historic control animals of this same strain is suggestive of a causative role for benzene.

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment  
01-JUL-2005 (424)

**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** 15 weeks  
**Frequency of treatment:** 4 hours per day/5 days per week/7 weeks and then 7 hours per day/5 days per week/8 weeks  
**Post exposure period:** until spontaneous death  
**Doses:** 200 ppm (converts to 0.65 mg/l); 70 males and 59 females  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 158 male and 149 female rats exposed in utero (as 12-day-old embryos) and postnatally to air for a total of 104 weeks. Twelve-day-old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 8 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning. Benzene concentration analysed by GC.

Significant methodological deficiencies  
no data about analytical concentration, one concentration tested, controls not sham-exposed for the same period, low number of animals (minimal 21/sex in treatment or 11/sex in control group) for final determination of specific tumor incidence, no statistical evaluation, limited histopathology.

The US Environmental Protection Agency (EPA) Gene-Tox Carcinogenesis Panel (Nesnow et al. 1986) reevaluated the raw data from these studies including the slides used for histopathology determinations. Their comment on this Maltoni study was that it was inconclusive (without giving any detailed reasoning).  
To the German Rapporteur, their decision seems to be explainable since the study design, data evaluation and documentation were not compliant to standard carcinogenicity bioassays. The target organs showed consistency to those of other studies, therefore the rapporteur interpreted the data from the Maltoni studies to give supportive evidence on benzene carcinogenicity via the inhalation route.

**Result:** No effects were seen on survival rate or body weight. When the sexes were considered both individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 3.9 compared with 0.7% in the control group), oral cavity carcinomas (incidence = 19 compared with 0%), nasal cavity carcinomas (incidence = 15

compared with 2%), hepatomas (incidence= 5.7 compared with 0.3%) and total malignant tumours (incidence = 31.4, 51.7 and 40.6% in the treated males, females and when combined respectively compared with 18.5, 17.6 and 18.0 in the control groups respectively). In the females, alone, increases were seen in malignant mammary tumours (incidence = 13.8 compared with 5.4%) and haemolymphoreticular neoplasias other than thymomas (incidence = 6.9 compared with 0.7%).

Tumor incidences in male and female offspring treated for 15 weeks followed by an observation period until week 150:  
No. of animals with tumors were related to the No. of animals at study begin:

Zymbal gland carcinoma:  
offspring males 4/70 vs 2/158 controls  
offspring females 1/59 vs 0/149 controls

oral cavity carcinomas:  
offspring males 2/70 vs 0/158 controls  
offspring females 6/59 vs 0/149 controls

nasal cavity carcinomas:  
offspring males 1/70 vs 0/158 controls  
offspring females 1/59 vs 0/149 controls

hepatomas:  
offspring males 2/70 vs 1/158 controls  
offspring females 5/59 vs 0/149 controls

**Source:**

BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:**

99.93% pure.

**Flag:**

Risk Assessment

01-JUL-2005

(734) (735) (736) (819)

**Species:**

rat

**Sex:** female

**Strain:**

Sprague-Dawley

**Route of administration:**

inhalation

**Exposure period:**

104 weeks

**Frequency of treatment:**

see remarks

**Post exposure period:**

until spontaneous death

**Doses:**

200, 300 ppm (converts to 0.65, 0.98 mg/l); 54 rats

**Control Group:**

yes, concurrent no treatment

**Method:**

other

**GLP:**

no data

**Test substance:**

other TS

**Remark:**

Control group consisted of 60 female rats exposed from day 12 of pregnancy to air for a total of 104 weeks. The benzene-treated group was exposed, from day 12 of pregnancy, to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks and finally to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment

period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The offspring from these dams were used in the 12 day-old embryo in utero exposure studies reported in the same references. Benzene concentration measured by GC.

Significant methodological deficiencies  
no data about analytical concentration, one treatment group, one sex, no statistical evaluation, limited histopathology.

The US Environmental Protection Agency (EPA) Gene-Tox Carcinogenesis Panel (Nesnow et al. 1986) reevaluated the raw data from these studies including the slides used for histopathology determinations. Their comment on this Maltoni study was that it was inconclusive (without giving any detailed reasoning).

To the German Rapporteur, their decision seems to be explainable since the study design, data evaluation and documentation were not compliant to standard carcinogenicity bioassays. The target organs showed consistency to those of other studies, therefore the rapporteur interpreted the data from the Maltoni studies to give supportive evidence on benzene carcinogenicity via the inhalation route.

**Result:**

The survival rate was slightly decreased after 118 weeks but not 104 weeks. No effect on body weight was noted. An increase was seen in Zymbal gland carcinomas (incidence = 5.7 compared with 1.7% in the control group), malignant mammary tumours (incidence = 11.1 compared with 3.3%) and total malignant tumours (incidence = 29.6 compared with 16.7%). Hepatomas and oral and nasal cavity carcinomas were slightly increased, being seen in one, two and one animals respectively and in none of the controls (incidence = 2.1, 40.0 and 16.7% respectively).

Increase of tumor incidences in animals treated for 104 Weeks at the end of follow up at week 150 (Maltoni et al., 1985):  
No. of animals with tumors were related to the No. of animals at study begin:

Zymbal gland carcinoma:  
adult females (parent) 3/54 vs 1/60 controls

oral cavity carcinomas:  
adult females (parent) 2/54 vs 0/60 controls

nasal cavity carcinomas:  
adult females (parent) 1/54 vs 0/60 controls

skin carcinomas:  
adult females (parent) 0/54 vs 0/60 controls

forestomach carcinomas:  
adult females (parent) 0/65 vs 0/149 controls

hepatomas:  
adult females (parent) 1/54 vs 0/60 controls

hemolymphoreticular neoplasia:  
adult females (parent): 0/54 vs 2/158 controls

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:** 99.93% pure.

**Flag:** Risk Assessment

01-JUL-2005 (734) (735) (736) (819)

**Species:** rat **Sex:** male/female

**Strain:** Sprague-Dawley

**Route of administration:** inhalation

**Exposure period:** 104 weeks

**Frequency of treatment:** see remarks

**Post exposure period:** until spontaneous death

**Doses:** 200, 300 ppm (converts to 0.65, 0.98 mg/l); 75 male and 65 female rats

**Control Group:** yes, concurrent vehicle

**Method:** other

**GLP:** no data

**Test substance:** other TS

**Remark:** Control group consisted of 158 male and 149 female rats exposed in utero (as 12-day-old embryos) and postnatally to air for a total of 104 weeks. Twelve-day-old embryos were exposed in utero and postnatally to 0.65 mg/l, 4 hours/day, 5 days/week for 7 weeks and then for 7 hours/day, 5 days/week for 12 weeks followed finally by exposure to 0.98 mg/l, 7 hours/day, 5 days/week for 85 weeks. The animals were weighed every 2 weeks during the treatment period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal and histological examination made of the major organs. The rats may also have had some oral exposure through their mothers' milk at weaning. Benzene concentration measured by GC. Significant methodological deficiencies one treatment group, no data about analytical concentration, low number of animals (minimal 5/sex in treatment group) for final determination of specific tumor incidence, no statistical evaluation, limited histopathology.

The US Environmental Protection Agency (EPA) Gene-Tox Carcinogenesis Panel (Nesnow et al. 1986) reevaluated the raw data from these studies including the slides used for histopathology determinations. Their comment on this Maltoni study was that it was inconclusive (without giving any detailed reasoning).

To the German Rapporteur, their decision seems to be explainable since the study design, data evaluation and documentation were not compliant to standard carcinogenicity bioassays. The target organs showed consistency to those of other studies, therefore the rapporteur interpreted the data from the Maltoni studies to give supportive evidence on benzene carcinogenicity via the inhalation route.

**Result:** Decreased survival rate and mean body weights were noted in both the treated males and females after 104 weeks. An

interim report notes a decrease in the numbers of white blood cells, due mainly to lymphocytopenia, after 91 weeks of the study. When the males and females were considered separately and combined, increased incidences were seen in Zymbal gland carcinomas (combined incidence = 10 compared with 0.7% in the control), oral cavity carcinomas (incidence = 33.3 compared with 0%), nasal cavity carcinomas (incidence = 7.5 compared with 0%), hepatomas (incidence = 6.8 compared with 0.3%) and total malignant tumours (incidence = 56.4 compared with 18.0%). Leukaemias were increased in the males (incidence = 2.7 compared with 0.6%) and malignant mammary tumours (incidence = 13.8 compared with 5.4%) and forestomach carcinomas (incidence = 60 compared with 0%) increased in the females.

Increase of tumor incidences in animals treated for 104 Weeks at the end of follow up at week 150 (Maltoni et al., 1985):  
No. of animals with tumors were related to the No. of animals at study begin:

Zymbal gland carcinoma:

offspring males 6/75 vs 2/158 controls  
offspring females 8/65 vs 0/149 controls

oral cavity carcinomas:  
offspring males 1/75 vs 0/158 controls  
offspring females 10/65 vs 0/149 controls

nasal cavity carcinomas:  
offspring males 1/75 vs 0/158 controls  
offspring females 2/65 vs 0/149 controls

skin carcinomas:  
offspring males 1/75 vs 0/158 controls  
offspring females 1/65 vs 0/149 controls

forestomach carcinomas:  
offspring males 0/75 vs 0/158 controls  
offspring females 3/65 vs 1/149 controls

hepatomas:  
offspring males 2/75 vs 1/158 controls  
offspring females 7/65 vs 0/149 controls

hemolymphoreticular neoplasia:  
offspring males 6/75 vs 12/158 controls  
offspring females 0/65 vs 1/149 controls

**Source:**

BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:**

99.93% pure.

**Flag:**

Risk Assessment

01-JUL-2005

(734) (735) (736) (819)

**Species:** mouse **Sex:** male/female  
**Strain:** B6C3F1  
**Route of administration:** gavage  
**Exposure period:** 103 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 1 week  
**Doses:** 25, 50, 100 mg/kg bw/day; 50 mice/sex/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** yes  
**Test substance:** other TS

**Remark:** Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.

**Result:** See section 5.4 for general systemic effects. The survival rates were significantly decreased in the high-dosed male and female animals. Zymbal gland squamous cell carcinomas occurred in both sexes of mice with positive trends, the incidences in the mid- and high-dosed males and high-dosed females being significantly greater than in the controls (incidence = 0, 3, 10 and 54% in males and 0, 0, 3 and 10% in females for the four groups respectively). All carcinomas and in particular squamous cell carcinomas were seen with increased incidence in the preputial gland of male mice, the incidences of each in the mid- and high-dosed groups being significantly greater than in the controls (incidences = 0, 7, 3 and 8% for all carcinomas and 0, 11, 62 and 78% for squamous cell carcinomas in the four groups respectively). In the females, granulosa cell tumours and benign mixed tumours occurred with significant positive trends in the ovaries, those in the high-dosed and mid- and high-dosed animals respectively being significantly increased (incidences = 2, 2, 12 and 14% for granulosa cell tumours and 0, 2, 24 and 14% for benign mixed tumours for the four groups respectively). Carcinomas and carcinosarcomas of the mammary gland were seen in female mice, those in the mid- and high-dosed and high-dosed animals respectively being significant (incidences = 0, 4, 10 and 16% for carcinomas and 0, 0, 2 and 8% for carcinosarcomas). Harderian gland adenomas and carcinomas occurred in the males and females respectively, the incidences in all the treated males (0, 17, 27 and 22%) were significantly and in the high-dosed females (0, 0, 0, 8%) marginally greater than the controls. Increased dose-related incidences of alveolar/bronchiolar adenomas and carcinomas were seen in both sexes when the tumour types

were considered either separately or combined (combined incidences = 20, 33, 38 and 43% for males and 8, 12, 20 and 27% for females). The incidences of total malignant lymphomas were significantly increased in all the treated males and females (8, 21, 20 and 31% and 31, 56, 52 and 45% respectively). A significant positive trend in squamous cell papillomas of the forestomach occurred in both sexes of treated mice. The incidences, however, were not statistically significant, indicating a weak carcinogenic activity at this site. In the females, the incidences of hepatocellular adenomas in the low-dosed group (2, 18, 10 and 8%) and, when combined with hepatocellular carcinomas, in the low- and mid-dosed groups (8, 27, 26 and 14%) were significantly elevated over the controls. Pheochromocytomas of the adrenal gland were seen in significant excess in the mid-dosed males (incidence = 2, 2, 14 and 2%).

According to the definitions of the NTP, the authors concluded, that under the condition of these 2-year gavage study, there was clear evidence of carcinogenicity of benzene in male and female B6C3F1 mice

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
REPSOL PETROLEO, S.A. MADRID

**Test substance:** >99.7% pure.  
**Flag:** Risk Assessment

04-JUL-2005

(845)

**Species:** other: rat and mouse **Sex:** male/female  
**Strain:** other: Fischer 344 and B6C3F1  
**Route of administration:** gavage  
**Exposure period:** 2 years  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 0  
**Doses:** 0, 25, 50, 100, 200 mg/kg, 60 animals/group  
**Control Group:** yes, concurrent vehicle

**Method:** other: U.S. National Toxicology Program

**Year:** 1989

**GLP:** yes

**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Mean body weights of the top dose groups of male rats and of both sexes of mice were lower than those of the controls. Survivals of the top dose group of rats and mice of each sex were reduced; however, at week 92 for rats and week 91 for mice, survival was greater than 60% in all groups. Compound-related nonneoplastic or neoplastic effects on the hematopoietic system, Zymbal gland, forestomach, and adrenal gland were found both for rats and mice. Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenicity of benzene in rats and mice. In male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell carcinomas of the oral cavity, and squamous cell carcinomas of the skin. In female rats, benzene caused increased incidences of Zymbal

gland carcinomas and squamous cell carcinomas of the oral cavity. In male mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), and squamous cell carcinomas of the preputial gland. In female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and Zymbal gland squamous cell carcinomas. Dose-related lymphocytopenia was observed for rats and mice. See also NTP, 1986.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

01-JUL-2005

(539)

**Species:** mouse **Sex:** male/female  
**Strain:** Swiss  
**Route of administration:** gavage  
**Exposure period:** 78 weeks  
**Frequency of treatment:** 4-5 days/week  
**Post exposure period:** until spontaneous death  
**Doses:** 500 mg/kg bw/day; 40 mice/sex  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group of 40 mice of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the exposure period and every 8 weeks during the post exposure observation period. A full necropsy was conducted on each animal.

**Result:** No effects were seen on the survival of the benzene-treated animals but body weight, particularly in the males, was affected. When considering each sex individually and as a combined group, increased incidences were recorded for total benign and malignant tumours combined (combined incidence = 70% compared with 38.7% in the control group), total malignant tumours (incidence = 52.5% compared with 25%), lung adenomas (incidence = 22.5% compared with 7.5%), lung atypic adenomas (incidence = 15.0% compared with 1.2%), Zymbal gland dysplasias (incidence = 8.7% compared with 0%) and Zymbal gland carcinomas (incidence = 6.2% compared with 0%). Mammary carcinomas were increased in the females (incidence = 47.5% compared with 5%) and lung adenomatous hyperplasia and adenocarcinomas were increased in the males (incidences = 2.5% for each compared with 0% for each in the control group).

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

**Test substance:** German Rapporteur  
99.93% pure.  
**Flag:** Risk Assessment  
01-JUL-2005 (731)

**Species:** other: mouse and rat **Sex:** male/female  
**Strain:** other: RF/J, Swiss, Sprague-Dawley, Wistar  
**Route of administration:** other: gavage and inhalation  
**Exposure period:** 52-104 weeks  
**Frequency of treatment:** 4 to 7 hr/day, 5 days/week (inhalation) or 4 to 5 days/week (gavage)  
**Post exposure period:** 0 to 52 weeks  
**Doses:** inhalation, 0, 200, 300 ppm; gavage, 0, 50, 250, 500 mg/kg  
**Control Group:** yes, concurrent vehicle

**Method:** other: nonguideline  
**Year:** 1989  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Benzene is carcinogenic when administered by ingestion and by inhalation and that it cause tumors in the various tested animal models (Sprague-Dawley rats, Wistar rats, Swiss mice, and RF/J mice). Benzene is a multipotential carcinogen, as it produces a variety of neoplasias in one or more of the tested animal models, including Zymbal gland carcinomas, carcinomas of the oral cavity, nasal cavities, skin, forestomach, and mammary glands, as well as angiosarcomas of the liver, hemolymphoreticular neplasias and tumors of the lung. The experiments also indicated a clear-cut dose-response relationship in benzene carcinogenesis.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Flag:** Risk Assessment  
04-JUL-2005 (738)

**Species:** rat **Sex:** male/female  
**Strain:** Fischer 344  
**Route of administration:** gavage  
**Exposure period:** 103 weeks  
**Frequency of treatment:** 5 days/week  
**Post exposure period:** 1 week  
**Doses:** 50, 100, 200 mg/kg bw/day for males; 25, 50, 100 mg/kg bw/day for females; 50 rats/sex/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** yes  
**Test substance:** other TS

**Remark:** Benzene administered in corn oil. Blood was withdrawn from ten randomly preselected animals from each sex and dose group at 12, 15, 18 and 21 months. Blood was also taken from moribund animals before they were killed and all animals at terminal kill. Groups of ten animals/sex were

administered these same benzene doses for 51 weeks; blood was withdrawn at 0, 3, 6, 9 and 12 months; the animals were killed at 12 months and necropsies were performed. All animals were observed twice daily and weighed weekly for 13 weeks and monthly thereafter. All gross lesions and tissue masses were examined histologically together with all the major organs.

**Result:**

See section 5.4 for general systemic effects. The high-dosed male and mid- and high-dosed female groups had significantly decreased survival rates when compared to the control groups. The female control group, however, apparently had exceptionally good survival when compared with historic control data for the same strain and sex of animal. Zymbal gland carcinomas occurred in both the male and female treated rats with a significant positive trend, the incidences in the mid- and high-dosed males and all the dosed females being significantly greater than in the vehicle control group (incidences = 6, 13, 24 and 40% in males and 0, 13, 11 and 30% in females for the control and three dose groups respectively). A significant positive trend was seen for the number of benzene-treated rats with squamous cell papillomas or carcinomas of the palate, lip and tongue when considered either separately (in the case of the males) or combined (in both the males and females) (combined incidence = 2, 18, 32 and 46% in males and 2, 10, 24 and 20% in females for the four groups respectively). The individual or combined incidences of skin squamous cell papillomas and carcinomas were increased in the male rats with a significant positive trend, the incidences in the high-dosed animals being significantly greater than in the controls (incidence = 0, 4, 2 and 10% for squamous cell papillomas and 0, 10, 6 and 16% for squamous cell carcinomas in the four groups respectively). A significant positive trend was found for endometrial stromal polyps of the uterus, the high-dose incidence being significantly greater than in the controls (incidence = 14, 14, 14 and 28% for the four groups respectively).

See also Huff et al., 1989.

**Source:**

BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:**

>99.7% pure.

**Flag:**

Risk Assessment

04-JUL-2005

(843)

**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** gavage  
**Exposure period:** 52 weeks  
**Frequency of treatment:** 4-5 days/week  
**Post exposure period:** untill spontaneous death or killed at 144 weeks  
**Doses:** 50, 250 mg/kg bw/day; 30 and 35 rats/sex respectively  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 30 rats/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal with histological examination being carried out on all the major organs.

**Result:** Results were limited to tabulated data on the target organs published in multiple publications. The study used uncommon observation to the spontaneous death of animals. The experiment ended after 144 weeks. A dose-related decrease in survival was seen both during the exposure period and the postexposure observation period. In the females, a dose-related increase in Zymbal gland carcinomas (incidences = 0, 7.1 and 28.6% for the control, low- and high-dosed groups respectively) and mammary gland carcinomas (13.3, 14.3 and 25.0% respectively) were noted. Oral cavity tumours were also reported in the high-dosed females (0, 0 and 9.5% respectively). The high-dosed males and all dosed females had elevated haemolymphoreticular neoplasias (0, 0 and 16.7% for the males and 3.3, 7.1 and 3.4% for the females). There was one hepatoma and one subcutaneous angiosarcoma both occurring at the high-dose level. These tumours are rarely seen in this strain of animal. In general, the incidences of total malignant tumours were clearly and dose-relatedly greater in the treated when compared with the control animals (incidences = 4.5, 4.3 and 33.3% for males; 23.3, 35.7 and 72.4% for females and 15.4, 21.6 and 54.7% when the two groups were combined for control, low-dosed and high-dosed groups respectively).

There were slight variations in percentages between the data from different publications.

The results from Maltoni et al. (1989) were:

Zymbal gland carcinomas:  
 50/males: 0%                      50/females: 6.5%  
 250/males: 0%                      250/females: 22.9%  
 control/males: 0%                  control/females: 0%

leukaemia:  
 50/males: 0%                      50/females 6.7%

250/males: 11.4%      250/females: 2.9%  
control/males: 0%      control/females 3.3%

oral cavity carcinomas  
250/males: 0%      250/females: 5.7%  
control/males: 0%      control/females: 0%

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
REPSOL PETROLEO, S.A. MADRID

**Test substance:** 99.93% pure.

**Flag:** Risk Assessment

04-JUL-2005 (733) (734) (739)

**Species:** rat      **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** gavage  
**Exposure period:** 104 weeks  
**Frequency of treatment:** 4-5 days/week  
**Post exposure period:** until spontaneous death  
**Doses:** 500 mg/kg bw/day; 40 rats/sex  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 50 animals/sex administered olive oil. Benzene was administered in olive oil. During the experiment, the animals were examined every 2 weeks, weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. Complete autopsy was performed on each animal with histological examination being carried out on all the major organs.

**Result:** No details on survival were given however an interim report notes decreases in body weight and total red blood cell and total white blood cell counts after 92 weeks of the study, the latter being mainly due to lymphocytopenia. In the full term study, when the sexes were considered individually and combined, increases were seen in the incidences of Zymbal gland carcinomas (combined incidence = 43.0 compared with 1.1% in the controls), oral cavity carcinomas (incidence = 52.6 compared with 0%), nasal cavity carcinomas (incidence = 6.1 compared with 0%), forestomach dysplasias/acanthomas (incidence = 21.8 compared with 0%), liver angiosarcomas (incidence = 8.6 compared with 0%) and total malignant tumours (incidences = 174.4 compared with 26.7% in the males; 147.5 compared with 22.4% in the females and 160.8 compared with 24.5% when the two groups are combined). The males also showed increased skin carcinomas (incidence = 28.1 compared with 0%) and the females, forestomach carcinomas (incidence = 31.6 compared with 0%) and hepatocarcinomas (incidence = 7.1 compared with 0%); the latter being based on only one case. A number of other tumours were reported which are, apparently, rarely seen in historic control data for this breed and strain of rat, for example lung adenocarcinomas and soft tissue liposarcomas

were seen in one and two males respectively.

Slight differences in percentages of incidences to the publication of Maltoni et al., 1989:

Zymbal gland carcinomas

SD-males: 45% SD-females 40%  
SD-control males: 2% SD-control females:0%

leukemia:

SD-males: 2.5% SD-females: 7.5%  
SD-control males: 6% SD-control females: 2%

oral cavity carcinomas:

SD-males 52.55 SD-females: 50%  
SD-control males: 0% SD-control females:0%

forestomach carcinomas in situ:

SD-males:0% SD-females: 15%  
SD control males: 0% SD-control females: 0%

forestomach invasive carcinomas:

SD-males:2.5% SD-females: 0%  
SD-control males: 0% SD-control females:0%

skin carcinomas:

SD-males: 22.5% SD-females:0%  
SD-control males:0% SD-control females 2%

liver angiosarcomas:

SD-males:5% SD-females 7.5%  
SD-control males: 0% SD-control females. 0%

liver hepatomas:

SD-males: 7.5% SD-females: 2.5%  
SD-control males: 6% SD-control females: 0%

nasal cavity carcinomas:

SD-males: 7.5% SD-females:2.5%  
SD-control males: 0% SD-control females:0%

**Source:**

BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Test substance:**

99.93% pure.

**Flag:**

Risk Assessment

04-JUL-2005

(732) (733) (739)

**Species:** rat **Sex:** male/female  
**Strain:** Wistar  
**Route of administration:** gavage  
**Exposure period:** 104 weeks  
**Frequency of treatment:** 4-5 days/week  
**Post exposure period:** until spontaneous death  
**Doses:** 500 mg/kg bw/day; 40 rats/sex  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group of 40 rats of each sex administered olive oil. Benzene administered in olive oil. The status and behaviour of the animals were examined three times daily and they were examined clinically for gross changes every 2 weeks. The animals were weighed every 2 weeks during the experimental period and every 8 weeks during the postexposure observation period. A full necropsy was conducted on each animal.

**Result:** A higher mortality rate was seen in both sexes treated with benzene and body weight, particularly in the males, was affected. When considering each sex individually and as a combined group, increased incidences were recorded for total malignant tumours (combined incidence = 50% compared with 22.5% in the control group), Zymbal gland squamous cell carcinomas (incidence = 16.2% compared with 0%), oral cavity squamous cell carcinomas (incidence = 7.5% compared with 1.2%) and nasal cavity indifferntiated carcinomas (incidence = 3.7% compared with 0%).

**Source:** BP Chemicals Ltd LONDON  
Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.93% pure.

05-JUL-2005

(731)

**Species:** rat **Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** 86 weeks  
**Frequency of treatment:** 4-7 hours/day, 5 days/week  
**Doses:** 0, 200, 300 ppm  
**Control Group:** yes, concurrent vehicle

**Method:** other: nonguideline  
**Year:** 1982  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Continuous exposure by inhalation to 200-300 ppm of benzene, for 4-7 hours daily, 5 days weekly, causes the onset of zymbal gland carcinomas in male and female Sprague-Dawley rats.

**Source:** Deutsche Shell Chemie GmbH Eschborn

05-JUL-2005

(737)

**Species:** human**Sex:****Remark:** A letter to the Editor, criticizing a meta-analysis of Non-Hodgkin's Lymphoma (NHL) in petroleum workers published by Wong and Raabe in an earlier volume (JOEM (2000) 42: 554-568).

The letter suggests that the meta analysis lacked sensitivity, that the healthy worker effect masked the true incidence of NHL, and the characterization of benzene exposure was inadequate.

**Source:**  
12-SEP-2001

Wong and Raabe refute each of these claims in their reply.  
A.K. Mallett Surrey

(426)

**Species:** human**Sex:****Method:** Subjects

The cancer cases in this study were children born after 31 December 1959 and identified from the Danish Cancer Registry as having a diagnosis of leukemia, tumor of the CNS or malignant lymphoma when under the age of 15 yr during the period January 1968 - December 1991. A total of 1,989 children were included in the study. 5,506 control children were selected at random from the entire Danish population.

**Residential history**

Familal background, including the residential history of each family, was obtained from central government records.

**Exposure assessment**

The average concentrations of benzene and nitrogen dioxide (used as markers of traffic-related pollution) at the families addresses were assessed using the Operational Street Pollution Model. Historic information on urban layout, traffic patterns and meteorologic data were obtained from central records. The geographic distribution and temporal variation in background air pollution levels were estimated from measurements made during 1994-1995 at four sites in different parts of Denmark, and from information on changes in air pollution since 1960.

**Statistics**

The risk of childhood cancer was analysed according to traffic density and air pollution level at the residence during pregnancy and during childhood. Cumulated exposure was calculated from the date of birth until 12 months before the date of diagnosis, with an additional requirement of at least 6 months of follow-up exposure (ie cancers diagnosed before age 18 months were excluded).

The relative risk for childhood cancer were estimated by conditional logistic regression analysis. Exposure and traffic density estimates were log transformed to avoid

undue influence from outliers.

Validation of exposure assessment

The ambient- and personal exposure of 204 children to present-day levels of nitrogen dioxide was measured and compared with the estimated exposure obtained from modelling (calculated as described above).

**Result:**

The risk of developing leukemia or CNS tumors, and the risk of developing "all cancers", was not increased in the higher categories of exposure. In contrast, there was an association between in utero exposure to air pollutants and the risk of developing lymphoma. The exposure-response pattern showed a 25% increase when the benzene concentration doubled (p for trend = 0.06), and a 51% increase (p for trend = 0.05) for a doubling of nitrogen dioxide concentration. Analysis of disease by morphologic subtype showed that this increase was restricted to Hodgkin's disease.

The association between the risk of lymphomas and exposure during childhood was less consistent, with the highest category of benzene exposure showing a decreased risk, but the opposite trend was apparent for nitrogen dioxide. There was no significant relationship between the risk of Hodgkin's disease and exposure during childhood.

The measured values in the nitrogen dioxide validation exercise were approximately 81% of the calculated values. While this would have led to some small degree of misclassification of the exposure grouping of some individuals, it had no impact on the overall trends or relative risk estimates.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The authors note that the finding of a higher risk of non-Hodgkin's lymphoma among children exposed to benzene and nitrogen in utero was of borderline significance and may be a chance event due to the multiple statistical testing used. While this may reflect a causal relationship with high outdoor levels of traffic pollution, they concluded that the evidence allowed no firm conclusion at present.

25-APR-2002

(917)

**Species:** human**Sex:****Method:**

## Subjects

The cohort comprised men and women from a 672 factories in 12 Chinese cities who were occupationally exposed to benzene between 1972 and 1987. Industries and occupations included painting, printing and the manufacture of footwear, paint and other chemicals. An unexposed control group was assembled from workers employed between 1972 and 1987 from these and other industrial activities.

The study group comprised 74,828 benzene exposed and 35,805 unexposed workers. Benzene exposed workers (47% women) were followed for an average of 10.5 yr, unexposed subjects (40% women) for 11.7 yr.

## Exposure

Exposure was estimated from a standardized "job title dictionary", covering 60 job titles. Exposure for each job was estimated during 5 year calander periods using an algorithmic model. Available air measurements (n=8477) were assigned to one of 6 concentration ranges (from <1ppm to >50 ppm) for 7 calander periods (starting in 1949 and ending with 1985+).

## Follow-up

Subjects were followed for history of benzene toxicity, selected hematopoietic malignancies and other hematological disorders and vital status to 31 December 1987. Cause of death was generally obtained from employer/factory records or death certificates. Only 147 exposed and 90 unexposed workers ere lost to follow-up.

## Verification

Newly diagnosed hematopoietic disorders during the follow-up period were subject to independent histopathologic- and hematopathologic scrutiny. Classification of different types of leukemia, lymphoma and myelodysplastic change followed recognised international criteria, and were assigned without knowledge of the patients' benzene exposure status.

## Statistics

Analyses for mortality were made by internal comparison of disease rates in the exposed and unexposed groups using Poisson regression analysis (adjusted for age and sex) to give a rate ratio (RR). Dose-response was assessed with respect to duration and cumulative exposure to benzene.

**Result:**

63 cases of lymphohematopoietic malignancy and 18 other selected hematologic disorders (agranulocytosis, aplastic anemia, MDS) were found among the 74,828 benzene exposed workers. The unexposed controls had 13 cases of lymphohematopoietic malignancy from a total of 35,805 workers.

Risk was significantly elevated for all lymphohematopoietic malignancies combined (RR = 2.6), total malignant lymphomas

(RR = 3.5) and total leukemias (RR = 2.6).

AML was significantly elevated (RR = 3.1), with significant excess risks found also for aplastic anemia and myelodysplastic syndrome.

Risk of ANLL/MDS was significantly elevated among workers hired before 1972.

ANLL/MDS was elevated among coatings workers (RR = 4.2), rubber workers (RR = 6.1), chemical workers (RR = 4.5) and among those with mixed occupations (RR = 4.4). The risk of NHL was significantly increased among chemical workers (RR = 7.8).

The risk for ANLL and ANLL/MDS increased with increasing average exposure to benzene (RR = 3.2 for constant exposure <10ppm; RR = 7.1 for constant exposure >25 ppm). The risk of ANLL/MDS was strongly associated with increasing amounts of recent (but not historic) benzene exposure.

**Source:** A.K. Mallett Surrey

**Conclusion:** The study confirms earlier findings of increased risk for ANLL and aplastic anemia among benzene exposed workers, indicates that MDS is linked to benzene exposure, and suggests that benzene increases the risk for ANLL/MDS at lower exposures than has previously been reported.

25-APR-2002

(488)

**Species:** human

**Sex:**

**Method:** Subjects and methods

The current investigation included all petroleum workers from previous cohort mortality investigations conducted in the USA, the UK, Canada, Australia, Italy and Finland (identified from a MEDLINE search). Although the exposure patterns in each might not be identical, all workers were potentially exposed to benzene-containing petroleum liquids as well as other hydrocarbons.

The total cohort comprised 308,199 individuals of which 226,000 were employed in refineries, the remainder being involved in downstream (distribution) or upstream (crude oil recovery) operations.

A meta-analysis procedure was used to combine data from the individual studies. Data on observed and expected deaths from non-Hodgkin's lymphoma (NHL) in each individual study was critical to completion of the re-analysis. The statistical procedure consisted of summing-up the observed and expected deaths from NHL from the individual studies and of calculating the pooled or metastandardised mortality ratio. The authors state that this procedure treats each separate cohort as a separate metastratum in data summarisation, thus adjusting for individual studies while preserving the original adjustment using substrata specific to age, race and time period.

**Result:** For the combined cohort of 308,199 subjects, no increased mortality from NHL was apparent with a total of 506 NHL deaths observed compared with 561.68 expected. The standardised mortality ratio was 0.90 and the 95% CI was 0.82 - 0.98.

Analyses of more than 226,000 refinery workers were performed by type of facility and industrial process. No significant elevation of SMR was detected for any individual cohort. Stratum-specific standardisation of mortality ratios (95% CI in brackets) were 0.96 (0.86 - 1.07) for US refinery workers and 1.00 (0.75 - 1.31) for UK workers. The pooled SMR for all non-US refinery workers was 1.12 (0.90 - 1.37). The SMR for product (gasoline) distribution workers was 0.64 (0.50 - 0.82) and 0.68 (0.47 - 0.95) for crude oil workers.

An analysis by date of first exposure was performed, in recognition of a reduction in the US occupational exposure limit for benzene that occurred just prior to 1950. This included 13 US cohorts and 2 from the UK. No difference in NHL mortality was found, with SMRs for the total (US+UK) population of 0.99 (0.87 - 1.12) and 0.95 (0.79 - 1.14) for workers hired before or after 1950, respectively.

**Source:** A.K. Mallett Surrey

**Conclusion:** Results from individual, as well as the pooled analysis, indicated that petroleum workers were not at an increased risk of NHL as a result of their occupational exposure to benzene or other benzene-containing products. This conclusion was supported by cohort studies of workers in other industries who were exposed to benzene as well as by population-based case-control studies of NHL and occupational exposure.

18-MAR-2002

(1276)

**Species:** human

**Sex:**

**Remark:** The authors of this letter dispute the conclusion of Wong and Raabe (2000) that benzene is not a cause on non-Hodgkin's lymphoma (NHL) in refinery workers. They put forward the following reasons:

- (i) many individuals included in the study would have had only minimal exposure to benzene, making it difficult to detect a proven benzene effect;
- (ii) they suggest that a meta-analysis of the cohorts used by Wong and Raabe would not have shown an increase in AML, suggesting that the findings fro NHL may be suspect;
- (iii) a relationship between NHL and exposure to petroleum-derived benzene may have been masked by the healthy worker effect.

**Source:** A.K. Mallett Surrey

15-MAR-2002

(425)

**Species:** human **Sex:**

**Remark:** (English abstract from original article in German)

"The aim of this review was to evaluate the relationship between occupational exposure to benzene and the risk of developing malignant haematolymphopoietic tumours, in particular non-Hodgkin's lymphomas.

Taking into consideration toxicological and epidemiological data and the genotoxic mechanism of benzene, it can be concluded that this chemical may cause 'precursor cell lymphomas'. According to the WHO classification of malignant lymphomas, these would belong to the so-called 'precursor lymphoblastic lymphomas'. A causative role of benzene in 'peripheral lymphomas', such as follicular lymphomas or chronic B-cell lymphocytic leukemia, is not regarded as biologically plausible. The results of epidemiological studies are in agreement with what is known of the genotoxic mechanism of benzene.

Another important aspect is the assessment of the risk of developing (acute) benzene-induced leukemia. Taking into consideration inhalation and dermal exposure, different risk groups for a variety of cumulative benzene doses (ppm years) are discussed."

**Source:** A.K. Mallett Surrey  
15-MAR-2002

(516)

**Species:** human **Sex:**

**Remark:** This paper reviews published epidemiological data on the dose-response relationship between occupational exposure to benzene and the risk of lymphohaematopoietic malignancy. It focuses primarily on information from a cohort of highly-exposed US rubber workers ("Pliofilm cohort") and an epidemiological investigation in China (the National Cancer Institute/Chinese Academy of Preventive Medicine study, NCI/CAPM). It also clarifies methodologic aspects of the NCI/CAPM study.

Quantitative relationships for cancer risk from China and the US are tabulated and show a relatively smooth increase in risk for acute myeloid leukemia and related conditions over a broad range of benzene exposures: below 200 ppm-years mostly from the NCI/CAPM study, and above 200 ppm-years from the Pliofilm study.

Hayes et al. note that the NCI/CAPM study extended epidemiological investigations to benzene exposure levels not adequately investigated in the past. The results showed a significant excesses of acute non-lymphocytic leukemia/myelodysplastic syndrome (ANLL/MDS) at average exposures of less than 25 ppm, with evidence of a doubled risk at average exposures under 10 ppm. Concerns have been

raised, however, about the exposure methods that were applied to this population of Chinese workers. They conclude that these issues do not undermine the finding of significantly elevated risks of lymphoreticular disorders in Chinese workers at average exposures substantially below those assigned to the Pliofilm cohort.

**Source:** A.K. Mallett Surrey (487)  
17-MAR-2002

**Species:** human **Sex:**

**Remark:** This paper continues debate of the strengths and weaknesses of the health survey conducted by the Chinese Academy of Preventative Medicine on 528,729 benzene-exposed workers (the NCI-CAPM study).

It follows a published response by Hayes et al (2001, Am J Ind Med, 40, 117) to earlier comments from this author (Wong).

A key discussion point is the reliability of exposure estimates used to support the NCI-CAPM study. Wong notes that these were consistently and substantially lower than actual exposures, documented in Chinese occupational medicine journals, which show that these individuals were exposed to levels of benzene that exceeded the 40 mg/m<sup>3</sup> national standard in place in China at that time. Exposures in the range 50-500 mg/m<sup>3</sup> were said to be commonplace. Wong contrasts this information with a claim that the NCI-CAPM study was sufficiently reliable to extend risk estimates to levels of exposure below 10 ppm.

**Source:** A.K. Mallett Surrey (1275)  
04-MAR-2003

**Species:** human **Sex:**

**Remark:** This review provides a comprehensive analysis of metabolic and mechanistic factors believed to be involved in benzene-induced leukemia. It includes the following elements:

- \* Historical perspective
- \* Benzene toxicity
- \* Benzene and leukemia
- \* Animal carcinogenicity studies
- \* Interaction of benzene metabolites
- \* Species differences in metabolism
- \* Covalent binding to macromolecules
- \* Myelodysplastic syndrome
- \* Chromosomal damage
- \* Studies using myeloblasts in culture
- \* Mechanisms of toxicity/leukemogenesis
- \* Issues in benzene risk assessment

The author discusses uncertainties in our current

understanding of the complex mechanisms by which benzene induces leukemia. If this is a result of covalent binding of benzene metabolites to DNA it would be a non-threshold event, while other mechanisms, perhaps involving an alteration in protein functionality, would require some finite level of exposure and the dose-response curve would exhibit a threshold. Experimental results are available that support both mechanisms, however the author notes that evidence is emerging which indicates that specific chromosomal aberrations resulting from benzene-related changes in specific structural proteins or enzymes are more important than DNA adduct formation. The enzymes involved in benzene metabolism and detoxication, the control and function of interleukins in bone marrow by benzene metabolites, the control of apoptosis in bone marrow cells and the inhibition of topoisomerase II are all protein-mediated and part of the process of leukemogenesis. The most significant genotoxic event appears to be a series of chromosomal translocations, the mechanisms of which are not known. This movement, intact, of a segment of one chromosome to a sister chromosome is a highly ordered process that is unlikely an uncontrolled event and is probably protein mediated. Such considerations suggest that DNA damage preceding benzene-induced leukemogenesis is a protein-mediated threshold event.

**Source:**  
26-JAN-2003

A.K. Mallett Surrey

(1072)

**Species:**

human

**Sex:**

**Remark:**

Chinese language paper, translation unavailable:

ABSTRACT:

"The relationship between glutathione-S-transferase M1(GST.mu.) gene deletion and leukemia in workers exposed to benzene was studied. A matched population-based case-control survey with multivariate Logistic regression analysis was conducted in this study. In the population of 34 patients and their matched controls, the absence of the GST.mu. genotype conferred odds ratio of 3.6. It suggested that GST.mu. was an important determinant of heterogeneity in individual susceptibility to leukemia associated with exposure to benzene. The single-variance analysis indicated that these markedly significant factors were GSF.mu. gene deletion, GSF.mu. isoenzyme in workshop air. The multivariate anal. indicated that these markedly significant factors were GSF.mu. gene deletion, duration of exposure to benzene and GSF.mu. isoenzyme activity."

**Source:**  
**Conclusion:**

A.K. Mallett Surrey

GSF.mu gene deletion may be associated with increased risk of leukemia in workers exposed to benzene.

04-MAR-2003

(1232)

**Species:** human**Sex:****Method:**

## SUBJECTS AND METHODS

The study population was drawn from a cohort of 170,000 men employed by Electricite de France-Gaz de France (EDF-GDF) for at least one year between 1978 and 1989. All incident cases of leukaemia (ICD-9 204-208) diagnosed in the cohort in the period 1978-1989, and still active in the company at the time of diagnosis, were included in the 'cases' (n=72). Four age-matched controls per case were selected at random from other active, non-retired EDF-GDF workers (n=285). Socio economic status was determined according from the first job the subject held with EDF-GDF, according to the French classification of social class.

Note: Only currently employed workers were available for inclusion in this study, hence cases of leukemia diagnosed after workers had retired from EDF-GDF were not accessible to the researchers. This resulted in a fairly young study population (approx. 45 yr).

## ASSESSMENT OF OCCUPATIONAL EXPOSURE

Occupational exposure to chemicals, physical agents or industrial processes categorised by IARC proven-, probable- or possible carcinogens (Group 1, 2A or 2B, respectively) or "other exposures of particular interest" were identified. Exposures were evaluated by occupational health professionals employed in different sections of EDF-GDF, and were based upon a list of work tasks and frequency/duration of exposure. This information was used to estimate the proportion of working time that the subject was 'exposed' to each chemical, agent, process etc.

## BENZENE EXPOSURE ASSESSMENT

Benzene exposure was estimated on the basis of two regulations that limited its concentration in solvents, one in 1969 (benzene content <1%v/v) and one in 1986 (benzene content <0.2% w/w). Based on these assumptions, benzene exposure was said to have decreased 5-fold (for a given work task) between 1970-1985, and by a further factor of 5 after 1985. Gasoline-derived exposures were considered to be greater than those from solvents due to the higher benzene content of gasoline (said to be 5% in 1970). The estimates for frequency and duration of "use of solvents" or "exposure to gasoline" were combined with the time weighted average exposure to benzene (arbitrary unit years).

Exposures were stratified into 4 groups on the basis of arbitrary units :

- > 0 <1.1 arbitrary unit years
- >= 1.1 <5.5 arbitrary unit years
- >= 5.5 <16.8 arbitrary unit years
- >= 16.8 arbitrary unit years

## ANALYSIS

Individual cumulative exposures were calculated from the

date of start of employment at EDF-GDF to the date of cancer diagnosis (for controls, to the date of diagnosis of the matched case). To allow for latency, cumulative exposure was also assessed after excluding exposures during the last 2, 5 or 10 yr. EGRET software was used to perform conditional logistic regression on the data.

#### STATISTICAL METHODS

Univariate analysis of variance was initially performed on the data, followed by multivariate analyses including the exposure variables that had been associated with leukaemia in the univariate analysis.

**Remark:**

Since accurate information on benzene exposure was not available, the authors reported exposure information as "arbitrary" time-weighted average values i.e. unit years. These exposures were then translated by the authors into ppm values using further assumptions that are not described. When discussing these results, they state that published monitoring data leads to a conclusion that 1 unit is equivalent to 1 ppm, however this is based upon a single publication of unknown reliability. As a result, they predict that the median benzene exposure experienced would therefore be 0.16 ppm, with a median cumulative exposure of 1.1 ppm years. No independent validation of these assumptions is presented, however.

**Result:**

#### GENERAL

Unadjusted odds ratios indicated that the risk of leukaemia increased with cumulative exposure to benzene, reaching 3.6 in the highest exposure group (>16.8 ppm years) with a significant trend (P = 0.02). Unadjusted odds ratios also showed some evidence of an increased risk of leukaemia with exposure to asbestos (OR 2.5), chlorinated solvents (OR 2.7) and coal tars (OR 3.0) with significant trends.

Adjusted odds ratios for benzene were decreased slightly (3.1) however the trend was no longer significant (P = 0.30). Adjusted odds ratios for asbestos, chlorinated solvents and coal tars also decreased (0.6, 1.6, 1.9 respectively) with no significant trend. The authors note that "Strong correlations between the exposures may have led to over adjustment, making the odds ratios difficult to interpret."

#### LEUKEMIA RISKS RELATED TO BENZENE EXPOSURE

In their remaining analyses, only leukaemia risks linked to benzene exposure were presented.

When considering benzene exposure arising solely from solvents, the OR in the high exposure category was no longer statistically significant, the risks were slightly decreased and the trend test (P = 0.06) was just below the limit of significance. Too few subjects were exposed gasoline alone to permit detailed analysis of risk.

The risk of leukaemia was increased in workers with first exposure to benzene before 1960 (OR 2.1; trend non

significant). The risk also increased with an exposure duration of 10 (OR 2.0) or 20 years (OR 3.8), with a significant trend (P=0.02).

Exclusion of benzene exposures occurring during the 2, 5 or 10 years immediately preceding diagnosis of leukaemia gave odds ratios in a range 4.2-4.3 with a significant trend (P=0.01 to 0.02).

An odds ratio of 4.6 was obtained for all acute leukaemia in the highest exposure category, with elevated odds ratios for both myeloid- (OR 2.4) and lymphoid- (OR 3.3) sub-types.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The authors conclude there was an increased risk of acute leukaemia among subjects with an estimated median TWA exposure of 0.16 ppm. No detailed, historical exposure data were available for the population included in this study, however, and this exposure estimate was based upon generic published values. Hence while the incidence of leukaemia in this subpopulation appears increased, no conclusion is possible on the underlying dose-response relationship.

04-MAR-2003

(454)

**Species:**

human

**Sex:****Method:**

## SUBJECTS AND METHODS

The study cohort included 4172 men and 245 women employed between 1940 and 1977 at a petrochemicals in Sauget, Illinois. Vital status follow-up was completed for 4352 workers (99%), of which 2431 (56%) were deceased. Death certificates were obtained for 99% of decedents; those missing cause of death details were included in the all-cause mortality analysis. Worker death rates were compared with rates for the population of Illinois.

## BENZENE EXPOSURE ASSESSMENT

The history of process changes at the plant, area sampling levels, individual exposure data (1090 personal 8 hr TWA values and 247 personal short term exposure levels) and expert judgement from the plant's industrial hygienist were used to estimate exposure. Most of the exposures that were stratified by time, department and job had to be estimated since no relevant personal exposure data were available.

Continuous exposures were estimated as 8 hr TWA values using categories of <1 ppm, 1-10 ppm and 11-50 ppm. The number of days on which a 15 min exposure excursion of >100 ppm was also estimated along with estimated potential peak exposures for all jobs.

Cumulative individual daily exposures were divided into 3 exposure categories: < 1 ppm yr; 1-6 ppm yr; >6 ppm yr. Cumulative exposures were in a range 0.1 ppm yr to 632 ppm yr, with a median of 3 ppm yr. The cumulative number of days with predicted peak exposures of >100 ppm were divided into 4 exposure categories: no day over 100 ppm; <7 d; 7-40 d;

>40 d. The number of days with peak exposure >100 ppm was in a range 1-2590 d, with a median of 22 days. Most job types had no potential for peak exposures >100 ppm.

Comment: The authors note that only limited information was available to estimate benzene exposures during the 1940s and 1950s, and exposures may have been higher than predicted. This may have introduced bias into risk estimates associated with either cumulative or peak exposures.

#### STATISTICAL METHODS

Standardised mortality ratios (SMRs) and 95% CI were calculated using the Fisher exact test.

**Result:**

There was little evidence of increasing risk with increasing cumulative exposure for all leukemias or acute non-lymphocytic leukaemia (ANL), or other lymphohaematopoietic cancers with the exception of multiple myeloma, where the following SMRs were obtained:

Non-exposed group: 1.1 (95% CI = 0.3-2.5)  
<1 ppm yr: 1.4 (95% CI = 0.2-5.1)  
1-6 ppm yr: 1.5 (95% CI = 0.2-5.4)  
>6 ppm yr: 2.6 (95% CI = 0.7-6.7)

When peak exposure to 100 ppm for >40 d/yr were considered, the number of multiple myeloma (SMR 4.0, 95% CI = 0.8-11.7), all leukemias (SMR 2.7, 95% CI 0.8-6.4) and ANL (SMR 4.1, 95% CI 0.5-14.9) were greater than expected.

Ten of the 13 deaths from multiple myeloma occurred among workers with benzene exposure (8 with cumulative exposure, 2 with cumulative and peak exposure, 2 with only peak exposure). All 10 deaths occurred 20 or more years after first exposure (SMR 1.8, 95% CI 0.9-3.3).

No deaths from anaemia were found among the workers.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

While the number of deaths observed in the study was small, increased rates of ANL and multiple myeloma were found at benzene exposures >100 ppm, with high and intermittent exposures presenting the greatest risk. The number of peak exposures >100 ppm benzene appeared to be a better predictor of risk than cumulative exposure.

16-MAR-2004

(227)

**Species:** human**Sex:****Method:**

## SUBJECTS AND METHODS

This report is an update for a cohort of rubber hydrochloride workers that had previously demonstrated an association between benzene exposure and an excess of leukaemia. It covers a 15 year period (1981-1996) since the last update (Rinsky et al. (1987) *New Eng J med*, 315, 1044). The SMR analysis included in the current update included all 1,291 persons with a least 1 ppm-day exposure of exposure between 1 January 1940 and 31 April 1976. Exposure-disease relationships were also modelled for 1,845 non-salaried workers (1,291 exposed and 554 unexposed) who were alive as of 1 January 1950. Background to this study has been published before (Rinsky et al. (1981) *Am J Ind Med* 2, 217; Rinsky et al. (1987) as above).

## MODELLING

A generalised form of the Cox proportional hazards regression model was used to evaluate the effects of benzene on the risk of leukaemia and multiple myeloma. All workers employed for at least one day in the rubber hydrochloride departments after 1 January 1950 and alive as on 1 January 1950 were included. Total benzene exposure was estimated from duration of exposure and by accumulated ppm-years. Benzene exposure was also treated as a dichotomous variable where workers with at least 1 ppm-day exposure were compared with their unexposed counterparts. Lag periods of various lengths were applied to allow for an induction period between exposure and death.

**Result:**

Nine hundred and seventy six members of the 1,845 member cohort (including both exposed and unexposed) died between 1 January 1950 and 31 December 1996. Six hundred and seventy nine of the 1,291 subjects with at least 1 day's exposure to benzene also died during this period. In the 15 yr since the last published update, the proportion of deaths had nearly doubled (from 28.3% to 52.9%).

Mortality from all causes and from malignant neoplasms was similar to that of the US population, however deaths from lymphatic and haematopoietic neoplasms were elevated with a combined SMR of 1.64 (95% CI 1.06-2.41). The SMR for leukaemia for the whole cohort was 2.47 (95% CI 1.38-4.07) and for white males was 2.56 (95% CI 1.43-4.22). There were 17 leukemia deaths in the cohort, however two of these did not have the minimum 1 ppm-day exposure and so were excluded from the SMR analysis. The leukaemia cases had widely varying latency from first exposure, with 6 of the 15 exposed deaths occurring at least 30 yr after first exposure. In exposed white males, SMR for leukaemia increased with exposure category:

1 ppm-day to 30.99 ppm-yr:	1.45
40-199.99 ppm-yr:	3.21
200-399.99 ppm-yr:	5.55
>400 ppm-yr:	23.96

Summary SMRs for leukaemia in white males declined from 3.37 (95% CI 1.54-6.41) in the previous update to 2.56 (95% CI 1.43-4.22) in the current study.

The relationship between benzene exposure and leukaemia mortality appeared to be best modelled using a linear cumulative exposure with no lag.

Four new multiple myeloma deaths occurred since the 1981 update bringing the total to 8, however 3 of the 4 new cases were in workers judged to be unexposed and were not included in the SMR calculations. Of the remainder, 4 occurred in the lowest exposure category (1 ppm-day to 30.99 ppm-years of exposure) and the fifth was in the highest exposure category (>400 ppm-years).

**Source:** A.K. Mallett Surrey

**Conclusion:** The results reaffirm the leukemogenic effects of benzene exposure and suggest that excess risk had diminished with time (possibly reflecting a decline in benzene exposure).

19-MAR-2004

(942)

### **5.8.1 Toxicity to Fertility**

**Type:** other: Reproductive toxicity of vapor recovery unit gasoline.

**Species:** rat

**Sex:** male/female

**Strain:** Sprague-Dawley

**Route of administration:** inhalation

**Exposure Period:** 6 hr/day

**Frequency of treatment:** 6 hr/day, 7 days/wk

**Duration of test:** 10 wk exposure prior to mating. Females continued to be exposed until gestation day 20.

**Doses:** 0, 5000, 10000, 20000 mg/m<sup>3</sup>

**Control Group:** yes, concurrent no treatment

**Method:** other

**Year:** 2000

**GLP:** yes

**Test substance:** other TS: Vapor Recovery Unit Gasoline

**Remark:** Gasoline (CAS 86290-81-5) is one of the world's largest volume commercial products. Although numerous toxicology studies have been conducted, the potential for reproductive toxicity has not been directly assessed. Accordingly, a two-generation reproductive study in rats was conducted to provide base data for hazard assessment and risk characterization. The test material, vapor recovery unit gasoline (68514-15-8), is the volatile fraction of formulated gasoline and the material with which humans are most likely to come in contact. The study was of standard design. Exposures were by inhalation at target concentrations of 5000, 10000 and 20000 mg/m<sup>3</sup>. The highest exposure concentration was approximately 50% of the lower explosive limit and several orders of magnitude above anticipated exposure during refueling. There were no

treatment-related clinical or systemic effects in the parental animals and no microscopic changes other than hyaline droplet nephropathy in the kidneys of the male rats.

None of the reproductive parameters were affected and there were no deleterious effects on offspring survival and growth. The potential for endocrine modulation was also assessed by analysis of sperm count and quality as well as time to onset of developmental landmarks. No toxicologically important differences were found. Therefore, the NOAEL for reproductive toxicity in this study was greater than or equal to 20000 mg/m3. The only systemic effects, in the kidneys of the male rats, were consistent with an alpha-2-mu-globulin-mediated process. This is a male rat-specific effect and not relevant to human health risk assessment.

Additional Comments: Vapor recovery unit (VRU) gasoline, the volatile fraction of gasoline, was judged to be representative of the material to which humans are exposed. The VRU gasoline test sample was predominately (89% by volume) C4 and C5 aliphatic constituents and contained only 1.4 volume % aromatics. The two major aromatics, benzene and toluene, were present each at 0.7 volume % in the VRU gasoline test sample and benzene was found to be 0.7 volume % in the terminal vapor at the end of the study. The results of this study indicate that endocrine-mediated processes are not affected by VRU gasoline. There were no effects on fertility, sperm count, sperm quality, estrous cycling, quantitation of primordial oocytes, offspring sex ratio or body weight gain. There were no pathologic changes in reproductive organs and there were no differences in any specific landmarks related to sexual development in either generation. In summary, there were no effects in this study to suggest that VRU gasoline (comprised of mainly C4/C5 aliphatics and small amounts of benzene and toluene) alters hormone balance.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(754)

<b>Type:</b>	Fertility
<b>Species:</b>	rat
<b>Sex:</b>	female
<b>Strain:</b>	no data
<b>Route of administration:</b>	inhalation
<b>Exposure Period:</b>	10 to 15 days before cohabitation with males and 3 weeks after cohabitation (gestation)
<b>Frequency of treatment:</b>	daily/24 hours per day
<b>Premating Exposure Period</b>	
<b>female:</b>	10-15 days
<b>Duration of test:</b>	4.5-5 weeks
<b>Doses:</b>	0.001, 0.0065, 0.0204, 0.0566, 0.0633, 0.67 mg/L; group sizes unspecified
<b>Control Group:</b>	no data specified
<b>Method:</b>	other
<b>GLP:</b>	no data

**Test substance:** no data

**Remark:** Data about this study are taken from the English abstract of the original article in Russian and partly from secondary literature.

**Result:** Females were exposed continuously for 10-15 days before mating with untreated males and for 3 weeks after mating. A complete absence of litters was observed in female rats exposed to 0.67 mg/L (210 ppm), but not at the lower exposure levels. It is not known whether observations made at the 210 ppm level were due to failure to mate, infertility or early implantation losses of the fertilized ova. Whereas differences in individual organ weights of the dams were indicated for all exposure levels, any impairment of the newborn weight and/or induction of malformations was not reported for the lower exposure levels.

**Source:** German rapporteur; BP Chemicals Ltd LONDON

**Flag:** Risk Assessment

17-JUN-2005

(48) (419) (563)

**Type:** Fertility  
**Species:** rat  
**Sex:** female  
**Strain:** other  
**Route of administration:** inhalation  
**Exposure Period:** gestation days 6-13  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 21  
**Doses:** 0.15, 0.45, 1.5, 3.0 mg/l; groups of 20, 20, 22 and 22 rats respectively  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed fetuses noted. Fetuses and placentae were weighed and macroscopically examined. Half the fetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five fetuses and five dams were studied microscopically.

**Result:** No maternal deaths were seen at 0.15 mg/l, however 1-3 animals/ group died in the other treatment groups after completion of the exposures. Dissection revealed fresh haemorrhages in the lungs and kidneys and multiple incipient necroses in the liver, indicating ischaemia. Maternal weight gain and placental weight were statistically significantly decreased in all the treatment groups and liver/body weight ratio significantly increased at 0.45 mg/l and above. Increases were seen in the numbers of non-pregnant females at 1.5 and 3.0 mg/l and in the numbers of dams with total resorptions at 0.45 mg/l and above. Postimplantation foetal loss was statistically significantly

increased at 0.45 mg/l and above as a result of both early and late foetal mortality increases. See section 5.9 for developmental effects.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** p.a. grade benzene was tested.  
17-OCT-2000 (1112)

**Type:** Fertility  
**Species:** rat  
**Sex:** male  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure Period:** 10 weeks  
**Frequency of treatment:** 6hr/day, 5 days/week  
**Duration of test:** 20 weeks  
**Doses:** 30,300ppm  
**Control Group:** yes, concurrent no treatment

**Method:** other: no data  
**Year:** 1980  
**GLP:** no data  
**Test substance:** no data

**Result:** Testicular lessions.  
**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (105)

**Type:** Fertility  
**Species:** rat  
**Sex:** female  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure Period:** 15 D pre/1-22 preg  
**Frequency of treatment:** no data  
**Duration of test:** no data  
**Doses:** 670mg/m3/24h  
**Control Group:** no data specified

**Method:** other: no data specified  
**Year:** 1968  
**GLP:** no data  
**Test substance:** no data

**Result:** Effects on female fertility index  
**Source:** REPSOL PETROLEO, S.A. MADRID  
13-DEC-1996 (547)

**Type:** Fertility  
**Species:** rat  
**Sex:** female  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure Period:** 31-36 days  
**Frequency of treatment:** daily/24 hours per day  
**Premating Exposure Period**  
    **female:** 10-15 days  
**Duration of test:** 4.5-5 weeks  
**Doses:** 0.001, 0.0056, 0.0204, 0.0566, 0.0633, 0.67 mg/l;  
group size unspecified  
**Control Group:** yes

**Method:** other  
    **GLP:** no data  
**Test substance:** no data

**Remark:** Females were exposed continuously for 10-15 days before mating with untreated males and for 3 weeks after mating. Reliability: 4 (validity cannot be judged) data from secondary literature and partly from English abstract of the original article in Russian

**Result:** There was an absence of litters in the females but it is not known if this was due to failure to mate, infertility or early implantation losses of the fertilized ova.

**Source:** Deutsche Shell Chemie GmbH Eschborn

16-OCT-2000

(48) (419) (563)

**Type:** Fertility  
**Species:** rat  
**Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure Period:** during a 10 week pre-mating and mating period and from days 0 to 20 of gestation and from days 5 to 20 of lactation  
**Frequency of treatment:** during pre-mating and mating: 5 days/week, 6 hr/day; during gestation and lactation: daily, 6h/day  
**Premating Exposure Period**  
    **male:** no treatment  
    **female:** 10 weeks  
**Duration of test:** approximately 16 weeks  
**Doses:** 1, 10, 30, 300 ppm  
**Control Group:** yes, concurrent no treatment  
**NOAEL Parental:** 300 ppm

**Method:** other: nonguideline  
    **GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Including the highest tested concentration level of 300 ppm there were no effects on maternal body weight and body weight gain nor were there adverse effects on female fertility as measured by percentage pregnant animals, mean gestation length, number of litters, litter size, and viability of the pups. Thus, no effects on female

reproductive performance had been revealed. A trend toward reduced mean fetal body weights during the lactation period and towards reduced mean organ weights (testes, liver, kidney) on postnatal day 21 was observed at the 30 and 300 ppm level. Except for reduced body and liver weights in the female pups at 300 ppm, these changes were not statistically significantly different from controls. No treatment-related effects were seen in pup survival data during lactation or in the gross postmortem evaluation of these pups on Day 21 of lactation. Thus exposure of dams to benzene levels as high as 300 ppm resulted in only minimal effects on the pups.

NOAEL/rat,female: 300 ppm

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn;  
**Flag:** Risk Assessment  
17-JUN-2005 (646)

**Type:** Fertility  
**Species:** rabbit  
**Sex:** female  
**Strain:** New Zealand white  
**Route of administration:** inhalation  
**Exposure Period:** gestation days 6-19  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 29  
**Doses:** 0.5, 1.0 mg/l; 11 and 15 rabbits respectively  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavédelem, Munka-és Uzemegészségügy 29, 207-216, 1983.J. Hyg. Epidem. Microbiol. Immun. 24, 363-371, 1980.

**Result:** The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and a statistically significant increase in the number of dead or resorbed foetuses, mainly arising from abortion. At 0.5 mg/l, a slight non-significant decrease in maternal weight gain and increase in the number of dead or resorbed foetuses was reported.

**Source:** BP Chemicals Ltd LONDON  
17-OCT-2000 (1190)

**Type:** other  
**Species:** rat  
**Sex:** female  
**Strain:** other  
**Route of administration:** inhalation  
**Exposure Period:** up to 212 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** up to 212 days  
**Doses:** 0.28, 7, 14, 21, 30 mg/l; 10-25 rats/dose; low dose with both sexes  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

Reliability: 3 (not valid)

Unsuitable test system

no reproduction toxicity study, only additional comment

**Result:** See section 5.4 for general systemic effects. A moderate increase in testicular weight was seen in animals exposed for 93 days to 21 mg/l. No other effects on the testes were reported.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure.

16-OCT-2000

(1264)

**Type:** other  
**Species:** rat  
**Sex:** male/female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure Period:** 13 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Duration of test:** 13 weeks  
**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose  
**Control Group:** yes, concurrent vehicle  
**NOAEL Parental:** = 300 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 50 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 10 rats/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes

and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups sectioned and examined microscopically.  
Reliability: 3 (not valid)  
Unsuitable test system  
no reproduction toxicity study, only additional comment

**Result:** No exposure related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. No effects were recorded on the testes or ovary weights and no histological changes were seen on microscopic examination of the tissues from these organs.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** >99.9% pure.

24-OCT-2000

(1235)

**Type:** other  
**Species:** mouse  
**Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure Period:** gestation days 12-15  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 1.0 ml/kg bw (converts to 880 mg/kg bw); group size unspecified  
**Control Group:** no data specified  
**NOAEL Parental:** > 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene administered in cotton seed oil.

**Result:** A significant increase in maternal lethality and embryonic resorption was seen. Foetal weight was significantly reduced.

**Source:** BP Chemicals Ltd LONDON

24-OCT-2000

(816)

**Type:** other  
**Species:** mouse  
**Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure Period:** gestation days 6-15  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880 mg/kg bw/day); group sizes unspecified  
**Control Group:** no data specified  
**NOAEL Parental:** = 264 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene administered in cotton seed oil.

**Result:** At the mid- and high-dose levels, a significant increase in maternal lethality and in embryonic resorption was seen. Foetal weight was significantly reduced at all three doses.

**Source:** BP Chemicals Ltd LONDON  
24-OCT-2000 (816)

**Type:** other  
**Species:** mouse  
**Sex:** male/female  
**Strain:** CD-1  
**Route of administration:** inhalation  
**Exposure Period:** 13 weeks  
**Frequency of treatment:** 6 hours per day/5 days/per week  
**Duration of test:** 13 weeks  
**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 150 mice/sex/dose  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** Control group consisted of 150 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 20 mice/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups in the interim kills and from all treated groups at the final sacrifice sectioned and examined microscopically.

**Result:** No exposure-related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. A statistically significant, exposure-related decrease in the mean testis weight was seen at the top-dose on days 28, 56 and 91, corresponding to a decreased mean testis/body weight ratio on days 56 and 91. No effect on the ovary weight was noted. The top-dosed animals, dosed for the full 13 week period, were found to have moderate-moderately severe bilateral testicular atrophy/degeneration (7 mice), a moderate-moderately severe decrease in spermatozoa in the epididymal ducts (6 mice), a minimal-moderate increase in abnormal sperm forms (9 mice) and bilateral ovarian cysts (4 mice). Similar lesions, reported to be of doubtful biological significance, were seen in both sexes at lower doses [no details available]. Some animals tended to have decreased lymphoid infiltrates in many tissues including the testes.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** >99.9% pure.  
24-OCT-2000 (1235)

**Type:** other  
**Species:** mouse  
**Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure Period:** 269 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** up to 269 days  
**Doses:** 0.28 mg/l; 5-10 guinea pigs  
**Control Group:** no data specified

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

Reliability: 3 (not valid)

Unsuitable test system

no reproduction toxicity study, only additional comment

**Result:** See section 5.4 for general systemic effects. A slight increase in testicular weight was noted. No other testicular effects were reported.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** 99.98% pure.

24-OCT-2000

(1264)

**Type:** other  
**Species:** mouse  
**Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure Period:** 243 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** 243 days  
**Doses:** 0.26 mg/l; 1 or 2 rabbits  
**Control Group:** no data specified

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

Reliability: 3 (not valid)

Unsuitable test system

no reproduction toxicity study, only additional comment

**Result:** See section 5.4 for general systemic effects. Slight histopathological testicular changes (degeneration of the

germinal epithelium) were noted. It is difficult to draw any conclusions from this study due to low numbers of animals tested and the single dose level used.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** 99.98% pure.  
24-OCT-2000 (1264)

**Type:** other  
**Species:** rabbit  
**Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure Period:** 243 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** 243 days  
**Doses:** 0.26 mg/l; 1 or 2 rabbits  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

**Result:** See section 5.4 for general systemic effects. Slight histopathological testicular changes (degeneration of the germinal epithelium) were noted. It is difficult to draw any conclusions from this study due to low numbers of animals tested and the single dose level used.

**Source:** BP Chemicals Ltd LONDON  
**Test substance:** 99.98% pure.  
24-OCT-2000 (1264)

**Type:** other  
**Species:** rabbit  
**Sex:** female  
**Strain:** New Zealand white  
**Route of administration:** inhalation  
**Exposure Period:** 13 weeks  
**Frequency of treatment:** 6 hours per day/5 days/per week  
**Duration of test:** 13 weeks  
**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 100 mice/sex/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 100 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 20

mice/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups in the interim kills and from all treated groups at the final sacrifice sectioned and examined microscopically.  
Reliability: 3 (not valid)  
Unsuitable test system

**Result:** no reproduction toxicity study, only additional comment  
No exposure-related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. A statistically significant, exposure-related decrease in the mean testis weight was seen at the top-dose on days 28, 56 and 91, corresponding to a decreased mean testis/body weight ratio on days 56 and 91. No effect on the ovary weight was noted. The top-dosed animals, dosed for the full 13 week period, were found to have moderate-moderately severe bilateral testicular atrophy/degeneration (7 mice), a moderate-moderately severe decrease in spermatozoa in the epididymal ducts (6 mice), a minimal-moderate increase in abnormal sperm forms (9 mice) and bilateral ovarian cysts (4 mice). Similar lesions, reported to be of doubtful biological significance, were seen in both sexes at lower doses [no details available]. Some animals tended to have decreased lymphoid infiltrates in many tissues including the testes.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** >99.9% pure.  
24-OCT-2000 (1235)

**Type:** other  
**Species:** guinea pig  
**Sex:** male  
**Strain:** no data  
**Route of administration:** inhalation  
**Exposure Period:** 269 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** 269 days  
**Doses:** 0.28 mg/l; 5-10 guinea pigs  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

**Result:** See section 5.4 for general systemic effects. A slight increase in testicular weight was noted. No other testicular effects were reported.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** 99.98% pure.  
24-OCT-2000 (1264)

**Type:** other  
**Species:** other: rat, guinea pig, rabbit  
**Sex:** male  
**Strain:** other: rat Wistar, guinea pig unspecified, rabbit unspecified  
**Route of administration:** inhalation  
**Exposure Period:** rat: up to 136 days,  
guinea pig: up to 193 days  
rabbit: 175 days  
**Frequency of treatment:** 7 hours per day/5 days per week  
**Duration of test:** rat: up to 212 days  
guinea pig: up to 269 days  
rabbit: 243 days  
**Doses:** rat: 0.28, 7, 14, 21, 30 mg/l (converting to 88, 2200, 4400, 6600 and 9400 ppm); 10-25 animals/dose  
guinea pig: 0.28 mg/l (converting to 88 ppm); 5-10 animals/dose  
rabbit: 0.26 mg/l (converting to 80 ppm); 1-2 animals/dose  
**Control Group:** other: "air-exposed" and/or "unexposed" controls  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS: 99.98 % pure

**Remark:** All animals were weighed at regular intervals during the study and frequent observations made of their general appearance and behaviour. Records were kept of food consumption and mortality. Various organs, including the testes, were weighed and examined grossly and microscopically.

**Result:** See section 5.4 for general systemic effects.  
A slight to moderate increase in average testicular weight (no data given) was seen in rats after 93 days when exposed to 21 mg/l (6600 ppm) for 70 days and in guinea pigs after 269 days when exposed to 0.28 mg/l (88 ppm) for 193 days. Slight histopathological changes in testes (no data given) were observed in rabbits after 243 days when exposed to 0.26 mg/l (80 ppm) for 175 days.

**Source:** German rapporteur; BP Chemicals Ltd LONDON  
**Test substance:** 99.98% pure.  
**Flag:** Risk Assessment  
17-JUN-2005 (1265)

**Type:** other  
**Species:** other: rat, mouse  
**Sex:** male/female  
**Strain:** other: Sprague-Dawley (rat); CD-1 (mouse)  
**Route of administration:** inhalation  
**Exposure Period:** 13 weeks  
**Frequency of treatment:** 6 hours per day/5 days per week  
**Duration of test:** 13 weeks  
**Doses:** 1, 10, 30, 300 ppm (converts to 0.003, 0.033, 0.098, 0.98 mg/l); 50 rats/sex/dose; 150 mice/sex/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** other TS: >99.9% pure

**Remark:** rat:  
Control group consisted of 50 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56 and 91, 10 rats/sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during the study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups sectioned and examined microscopically.  
mouse:  
Control group consisted of 150 animals exposed to filtered air. All animals were observed twice daily for mortality and moribundity throughout the study and weekly for signs of toxicity. On days 0, 7, 14, 28, 56, and 91, 20 mice /sex/group were sacrificed and complete necropsies performed. Animals dying or sacrificed in a moribund condition during this study were also necropsied. The testes and ovaries were preserved in 10% neutral buffered formalin and those from the control and high-dosed groups in the interim kills and from all treated groups at the final sacrifice sectioned and examined microscopically.

**Result:** rat:  
No exposure related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects. No effects were recorded on the testes or ovary weights and no histological changes were seen on microscopic examination of the tissues from these organs.  
NOEL/rat: 300 ppm

mouse:  
No exposure related mortality, clinical observations or mean body weight changes were seen. See section 5.4 for explanation of other general systemic effects.  
A statistically significant, exposure-time related decrease in the mean testis weight was seen at the top-dose (300 ppm level) on days 28, 56 and 91, corresponding to a decreased mean testis/body weight ratio on days 56 and 91. No effects on the ovary weight was noted. The top-dose animals, dosed

for the full 13 week period, were found to have moderate to moderately severe bilateral testicular atrophy/degeneration (7 mice), a moderate to moderately severe decrease in spermatozoa in the epididymal duct (6 mice), a minimal to moderate increase in abnormal sperm forms (9 mice) and bilateral ovarian cysts (4 mice). Similar lesions were reported to be observed in both sexes also at lower dose levels (no data available). Some animals tended to have decreased lymphoid infiltrates in many tissues including the testes.

NOEL/mouse: 30 ppm

**Source:** German rapporteur; BP Chemicals Ltd LONDON

**Test substance:** >99.9% pure.

**Flag:** Risk Assessment

17-JUN-2005

(1235)

**Type:** other: human

**Method:**

Subjects and sample collection

The subjects were married workers employed for at least 1 yr in shoemaking, spray painting or paint manufacturing industries in Zhejiang province, with high exposure to benzene, toluene or xylene. Control were matched for age and occupation but had no BTX exposure. Semen and blood samples were obtained from 24 exposed workers and 37 non-exposed controls.

Semen analysis

Liquefaction time, semen pH, sperm concentration, total sperm count, percentage vitality and sperm activity were assessed following WHO guidelines. Acrosin activity, seminal fructose concentration, gamma-glutamyltransferase activity and lactose dehydrogenase 4 activity were also determined. BTX concentrations were determined using headspace GC.

Blood analysis

BTX in blood was determined using headspace GC.

Statistics

Results were analysed using Chi square, Student's t-test and multiple regression techniques.

**Result:** Mean workplace exposure to benzene was 103.34 mg/m<sup>3</sup>.

Benzene was present in blood (13 out of 24 samples, 4.4 umol/l) and sperm (12 out of 17 samples, 1.85 umol/l) of the exposed workers, but not the controls.

Mean sperm activity, acrosin activity and LDH-C4 activity were significantly lower in exposed workers.

Sperm vitality and work history, sperm motility and work history, sperm concentration and benzene concentration in blood showed significant associations after analysis using two-way ANOVA. (Note: there was no significant difference in sperm concentration between exposed and control populations).

**Source:** A.K. Mallett Surrey  
**Conclusion:** The authors conclude that exposure to BTX could affect sperm and related parameters. However the finding of a relationship between sperm concentration and blood benzene appears inconsistent with their observation that there was no difference in sperm counts between the exposed and control populations.

25-APR-2002

(1286)

### 5.8.2 Developmental Toxicity/Teratogenicity

**Species:** rat **Sex:** female  
**Strain:** other  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-13  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 21  
**Doses:** 0.15, 0.45, 1.5, 3.0 mg/l/day; groups of 20, 20, 22, 22 rats respectively  
**Control Group:** yes, concurrent no treatment  
**NOAEL Teratogenicity:** = 3 mg/l

**Method:** other  
**GLP:** no data  
**Test substance:** other TS

**Remark:** CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed foetuses noted. Foetuses and placentae were weighed and macroscopically examined. Half the foetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five foetuses and five dams were studied microscopically.

**Result:** See section 5.8 for reproductive effects. Mean foetal weight was decreased in all the exposed groups, this being statistically significant, and half to two-thirds of the foetuses showed retarded development at 0.45 mg/l and above, as seen by weights of less than 3.3 g. Skeletal retardation (missing or poorly ossified sternbrae, bipartite vertebral centres and shortened 13th rib) were slightly increased at 0.15 mg/l, the incidence being significantly higher in the other treatment groups. There was an increasing non-significant trend in the incidence of skeletal anomalies (irregular, fused sternbrae and supernumerary ribs) on exposure to 1.5 and 3.0 mg/l. External malformations were seen in three of the 653 foetuses (one case of missing tail at 0.45 mg/l and 2 cases of syndactylia at 1.5 mg/l) and none of the controls. No increase over the control group was seen in internal malformations and three foetuses had skeletal malformations (one case of missing sacral vertebrae at 0.45 mg/l; one case of fused metacarpal and carpal bones at 1.5 mg/l and one case of missing orbita). The incidence of these malformations was not considered to have been increased and the investigators concluded that benzene was

not teratogenic.

**Source:** BP Chemicals Ltd LONDON

**Test substance:** Supplied by Reanal, Budapest; p.a. grade.  
24-OCT-2000 (1110)

**Species:** rat **Sex:** female

**Strain:** Sprague-Dawley

**Route of administration:** inhalation

**Exposure period:** gestation days 6-15

**Frequency of treatment:** daily/6 hours per day

**Duration of test:** gestation day 20

**Doses:** 1, 10, 40, 100 ppm/day (converts to 0.003, 0.033, 0.12, 0.33 mg/l/day); 40 rats/dose

**Control Group:** yes, concurrent no treatment

**NOAEL Maternal Toxicity:** = 100 ppm

**NOAEL Teratogenicity:** = 100 ppm

**Method:** other

**GLP:** no data

**Test substance:** no data

**Remark:** Two control groups consisting of 40 females were exposed to filtered room air. Animals were examined prior to, during and after daily exposure and weighed at intervals. On day 20 dams were sacrificed and necropsied. Foetuses were removed by caesarian section and weighed and measured. Numbers of corpora lutea/ovary, implantation sites, resorptions and live and dead foetuses were recorded for each dam. Foetuses were examined for gross external variations and either sectioned to examine for visceral abnormalities or examined for skeletal abnormalities. Benzene vapour concentrations were determined daily and the mean ranges by infrared and gas chromatographic analyses found to be 1.02, 8.36-11.02, 37.36-41.21 and 96.59-104.15 ppm (0.003, 0.028-0.036, 0.12-0.14 and 0.32-0.34 mg/l) for the four groups respectively.

**Result:** No deaths and no treatment-related clinical signs or gross pathology were noted in the dams and the pregnancy rate was within the expected range. Average maternal body weight gain was very slightly lower than the controls in the top-dose but this was not statistically significant. The average number of implantation sites, number of resorptions, resorption incidence and number of live foetuses were similar in all groups. A small, statistically significant decrease in the average male and female foetal body weight and non-significant, slight decrease in mean foetal body length was recorded at 0.33 mg/l. A slight, non-significant increase in dilation of the renal pelvis and ureters was seen in the lowest- and top-dose groups and a small, non-significant decrease in the average number of sternbrae and caudal vertebrae occurred at 0.33 mg/l. No benzene-induced skeletal variants or soft tissue anomalies were reported. The investigators concluded that benzene was slightly foetotoxic as shown by reduced foetal weights and lengths and a statistically non-significant delay in ossification of extremities and sternbrae at 0.33 mg/l.

**Source:** BP Chemicals Ltd LONDON

24-OCT-2000

(218)

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/7 hours per day  
**Duration of test:** gestation day 20  
**Doses:** 10, 50, 500 ppm (converts to 0.033, 0.16, 1.63 mg/l);  
18, 20, 19 rats respectively  
**Control Group:** yes, concurrent no treatment  
**NOAEL Teratogenicity:** = 50 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 17 females exposed to filtered room air. On day 20 females were sacrificed, caesarian sections were performed and numbers of ovarian corpora lutea, resorption sites and live and dead foetuses examined. Each foetus was examined externally and weighed. Whole-body transverse sections were made and examined microscopically for visceral changes. The skeleton was examined microscopically for anomalies and ossification. Blood samples were obtained on days 5 and 20 and erythrocyte and leukocyte counts determined. Benzene vapour concentrations were determined daily and the means found to be 9.75, 52.9 and 513.9 ppm (0.032, 0.17 and 1.70 mg/l) for the three groups respectively.

**Result:** No deaths, observable illness or signs of compound-induced toxicity were noted in the dams and the pregnancy rate was within the expected range. Maternal body weight was decreased in the mid- and high-dosed dams and transient changes in mean body weight gain were noted at various stages throughout the study at all doses. No treatment-related effects were seen in any of the haematology parameters, at necropsy or on the number of implantation sites, resorbed and dead foetuses, incidence of live foetuses or sex distribution of the foetuses. Statistically significant decreases in the mean crown-rump distance of the high-dosed foetuses and mean body weights of the mid- and high-dosed live foetuses were noted when compared with the controls. Skeletal variants (excencephaly, angulated ribs and ossification of the forefeet out of sequence in one, one and two rats respectively) were seen at 1.63 mg/l and evidence of lagging ossification was recorded in the rib cage and extremities of the mid-dosed group and, together with the skull, vertebral column and pelvic girdle, the high-dosed group. A significantly fewer number of caudals compared to the controls were seen at 1.63 mg/l and a dose-related decrease in the mean number of metatarsals and phalanges occurred. Visceral examination revealed slight dilation of the ventricles in the brain in five mid- and four high-dosed animals. The investigators concluded that benzene was foetotoxic to rats at concentrations of 0.16 and 1.63 mg/l

and it manifests a teratogenic potential at 1.63 mg/l.  
**Source:** BP Chemicals Ltd LONDON (645)  
24-OCT-2000

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** Days 6-15 gestation  
**Frequency of treatment:** 6h/day  
**Duration of test:** no data  
**Doses:** 300,2200  
**Control Group:** yes

**Method:** other: no data  
**Year:** 1978  
**GLP:** no data  
**Test substance:** no data

**Source:** REPSOL PETROLEO, S.A. MADRID (437)  
24-OCT-2000

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** Days 6-15 gestation  
**Frequency of treatment:** 6h/day  
**Duration of test:** 20 days gestation  
**Doses:** 0,1,10,40 and 100ppm  
**Control Group:** yes, concurrent no treatment

**Method:** other: no data  
**Year:** 1986  
**GLP:** no data  
**Test substance:** as prescribed by 1.1 - 1.4

**Source:** REPSOL PETROLEO, S.A. MADRID (609)  
24-OCT-2000

**Species:** rat **Sex:** female  
**Strain:** other  
**Route of administration:** inhalation  
**Exposure period:** gestation days 7-14 (the day of finding sperm in the vaginal smear was considered the first day of gestation)  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 21  
**Doses:** 0.15, 0.45, 1.5, 3.0 mg/l/day (converting to about 50, 150, 500 and 1000 ppm); groups of 20, 20, 22, 22 rats respectively  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** other TS: benzene p.a. grade, supplied by Reanal, Budapest

**Remark:** CFY strain rats were used. Control group consisted of 48 females exposed to clean air. Body weights were recorded weekly. On day 21 of gestation, dams were sacrificed, caesarean sections performed and number and position of live, dead and resorbed fetuses noted. Fetuses and placentae were weighed and macroscopically examined. Half the fetuses were dissected under the microscope while the other half were investigated for skeletal abnormalities. In each group, the organs of five fetuses and five dams were studied microscopically.  
Benzene concentrations measured by GC.

**Result:** At 1.5 and 3.0 mg/l (500 and 1000 ppm) an increase was seen in the number of non-pregnant females. Also, there was a clear increase in the number of dams with total resorptions at 0.45 mg/l (150 ppm) and above, whereas there were no changes in the mean number of implantations per dams. Postimplantation fetal loss was statistically significantly increased to about 30 % and more at 0.45 mg/l (150 ppm) and above as a result of both early and late fetal mortality increases.  
Fetal evaluation revealed that average foetal body weight was decreased in all the exposed groups, this being statistically significant. Half to two-thirds of the fetuses showed growth retardation at 0.45 mg/l (150 ppm) and above, as indicated by fetal body weights of less than 3.3 g. Skeletal retardation (missing or poorly ossified sternbrae, bipartite vertebral centres and shortened 13th rib) were slightly increased at 0.15 mg/l (50 ppm), the incidence being statistically significantly higher in the other treatment groups. There was an increasing non-significant trend in the incidence of skeletal anomalies (irregular, fused sternbrae and supernumerary ribs) on exposure to 1.5 and 3.0 mg/l (500 and 1000 ppm).  
External malformations were seen in three of the 653 fetuses (one case of missing tail at 0.45 mg/l [150 ppm] and 2 cases of syndactylia at 1.5 mg/l [500 ppm]) and in none of the controls. No increase in comparison to the control group was seen in internal malformations; three fetuses had skeletal malformations (one case of missing

sacral vertebrae at 0.45 mg/l [150 ppm]; one case of fused metacarpal and carpal bones at 1.5 mg/l [500 ppm] and one case of missing orbita). The incidence of these malformations was not considered to have been increased and the investigators concluded that benzene was not teratogenic.

No maternal deaths were seen at 0.15 mg/l (50 ppm), however 1-3 animals/ group died in the other treatment groups after completion of the exposures. Dissection revealed fresh haemorrhages in the lungs and kidneys and multiple incipient necroses in the liver, indicating ischaemia. Maternal weight gain and placental weight were statistically significantly decreased in all the treatment groups and liver/body weight ratio significantly increased at 0.45 mg/l (150 ppm) and above.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn

**Test substance:** Supplied by Reanal, Budapest; p.a. grade.

**Flag:** Risk Assessment

17-JUN-2005

(1111)

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** gestation day 20  
**Doses:** 1, 10, 40, 100 ppm/day (converts to 0.003, 0.033, 0.12, 0.33 mg/l); 40 rats/dose used  
**Control Group:** yes, concurrent vehicle  
**NOAEL Maternal Toxicity:** = 100 ppm  
**NOAEL Teratogenicity:** = 100 ppm

**Method:** other

**GLP:** no data

**Test substance:** other TS: technical grade benzene, purchased from API

**Remark:** Two control groups consisting of 40 dams were exposed to filtered room air. Animals were examined prior to, during and after daily exposure and weighed at intervals. On day 20 dams were sacrificed and necropsied. Foetuses were removed by caesarian section and weighed and measured. Numbers of corpora lutea/ovary, implantation sites, resorptions and live and dead foetuses were recorded for each dam. Foetuses were examined for gross external variations and either sectioned to examine for visceral abnormalities or examined for skeletal abnormalities. Benzene vapour concentrations were determined daily and the mean ranges by infrared and gas chromatographic analyses found to be 1.02, 8.36-11.02, 37.36-41.21 and 96.59-104.15 ppm (0.003, 0.028-0.036, 0.12-0.14 and 0.32-0.34 mg/l) for the four groups respectively. Benzene chamber concentration variability ranged from 3-13%.

**Result:** No deaths and no treatment-related clinical signs or gross pathology were noted in the dams and the pregnancy rate was within the expected range. Average maternal body weight gain was very slightly lower than the controls in the top-dose but this was not statistically significant. The

average number of implantation sites, number of resorptions, resorption incidence and number of live fetuses were similar in all groups. A small, but statistically significant decrease in the average male and female foetal body weight of 6% and a non-significant, slight decrease in mean foetal body length was recorded at 0.33 mg/l (100 ppm) coinciding with a statistically non-significant delay in ossification of extremities and sternebrae. A slight, non-significant increase in dilation of the renal pelvis and ureters was seen in the lowest- and top-dose groups and a small, non-significant decrease in the average number of sternebrae and of caudal vertebrae occurred at 0.33 mg/l (100 ppm). No benzene-induced skeletal variants or soft tissue anomalies were reported. The investigators concluded that benzene was slightly foetotoxic as shown by reduced foetal weights and lengths and a statistically non-significant delay in ossification of extremities and sternebrae at 0.33 mg/l (100 ppm).

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn;  
**Flag:** Risk Assessment

17-JUN-2005

(219)

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/7 hours per day  
**Duration of test:** gestation day 20  
**Doses:** 10, 50, 500 ppm (converts to 0.033, 0.16, 1.63 mg/l);  
18, 20, 19 rats respectively  
**Control Group:** yes, concurrent vehicle  
**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 17 females exposed to filtered room air. On day 20 females were sacrificed, caesarian sections were performed and numbers of ovarian corpora lutea, resorption sites and live and dead fetuses examined. Each foetus was examined externally and weighed. Whole-body transverse sections were made and examined microscopically for visceral changes (1/3 of fetuses/group). The skeleton of the remaining fetuses were examined microscopically for anomalies and ossification. Maternal blood samples were obtained on days 5 and 20 and erythrocyte and leukocyte counts determined.

Benzene vapour concentrations were determined daily by GC analysis and the means found to be 9.75, 52.9 and 513.9 ppm (0.032, 0.17 and 1.70 mg/l) for the three groups respectively (variation in concentration max. 10%).  
**Result:** No deaths, observable illness or signs of compound-induced toxicity were noted in the dams and the pregnancy rate was within the expected range. Maternal body weight was significantly decreased in the mid- and high-dosed dams at day 15 but not at day 20, and significantly increased in the low-dose group at day 20. Transient changes in mean body

weight gain were noted at various stages throughout the study at all doses. No treatment-related effects were seen in any of the haematology parameters. No abnormalities were observed at necropsy that could be attributed to benzene exposure. The number of implantation sites, resorbed and dead fetuses, incidence of live fetuses or sex distribution of the fetuses were comparable among treated and control groups.

Statistically significant decreases in the mean crown-rump distance of the high-dosed fetuses (500 ppm) and of mean body weights of the mid- (50 ppm) and high-dosed (500 ppm) live fetuses were noted, but not at the 10 ppm level, when compared with the controls. Skeletal variants and abnormalities were found in single fetuses from four litters of the high-dose (500 ppm) level (exencephaly and angulated ribs in one rat respectively and ossification of the forefeet out of sequence in two rats). Evidence of lagging ossification was recorded in the rib cage and in extremities of fetuses of the mid-dosed (50 ppm) group and also in fetuses of the high-dose (500 ppm) group together with lagging ossification of skull, vertebral column and pelvic girdle. A significantly fewer number of caudals compared to the controls were seen at 1.63 mg/l (500 ppm), and a dose-related decrease in the mean number of metatarsals and phalanges occurred. Visceral examination revealed slight dilation of the ventricles in the brain in five mid- and four high-dosed animals.

The investigators concluded that benzene was foetotoxic to rats at concentrations of 0.16 (50 ppm) and 1.63 mg/l (500 ppm).

Incidental findings of abnormalities at the 500 ppm level were taken as some indication for a teratogenic potential.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn;  
**Flag:** Risk Assessment

17-JUN-2005

(645)

**Species:** rat **Sex:** female  
**Strain:** Sprague-Dawley  
**Route of administration:** inhalation  
**Exposure period:** days 6-15  
**Frequency of treatment:** 6 hr/day  
**Duration of test:** 21 days  
**Doses:** 0, 100, 300, 2200 ppm  
**Control Group:** yes, concurrent vehicle  
**NOAEL Maternal Toxicity:** = 300 ppm  
**NOAEL Teratogenicity:** = 2200 ppm

**Method:** other: nonguideline  
**GLP:** no data  
**Test substance:** no data

**Result:** Maternal toxicity as indicated by transient exposure-confined lethargy and by a decrease in maternal weight gain was evident only at the high concentration level of 2200 ppm. Implantation sites/litter, live fetuses/litter, percentage absorptions/implantations sites, percentage of litters with absorptions, and number of litters totally

absorbed were comparable between controls and exposed groups even at the high concentration of 2200 ppm. Fetal body weight and fetal crown-rump length was similar to controls at concentrations of 100 and 300 ppm, but was significantly reduced at 2200 ppm.

Soft tissue examination revealed no significant increase in the incidence of anomalies among the exposed animals. Skeletal examinations showed a statistically significant increase in the number of fetuses with delayed ossification in the 300- and 2200- ppm groups. The litter incidence of missing sternbrae was significantly increased at the 100 and 2200 ppm exposure groups. The female offspring appeared to be affected to a greater extent than males with respect to delayed ossification and missing sternbrae.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn  
**Flag:** Risk Assessment

17-JUN-2005

(438)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** gestation day 16  
**Doses:** 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5-10 mice/dose  
**Control Group:** yes, concurrent no treatment  
**NOAEL Teratogenicity:** = 20 ppm

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed pregnant mice were sacrificed, their uteri removed and the number of live, dead and resorbed fetuses recorded. Two male and two female fetuses/litter were weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells.

**Result:** Litter size, foetal weights and the numbers of dead, resorbed and malformed fetuses were within the control limits. In the fetuses, changes in the numbers of both erythroid precursor cells (i.e. colony forming unit-erythroid; CFU-E; and burst forming unit-erythroid; BFU-E) were observed, the more mature, CFU-E, cells being affected to a greater extent by all exposure concentrations. The number of foetal CFU-E cells were increased at 0.017 and 0.033 mg/l and decreased at 0.066 mg/l.

**Source:** BP Chemicals Ltd LONDON

25-OCT-2000

(607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 2-day-old neonates  
**Doses:** 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5-10 mice/dose  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. Two male and two female neonates/litter were selected at 2 days of age, weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells.

**Result:** No difference in CFU-E numbers were noted between the low-dose offspring and the controls. Offspring exposed, in utero, to 0.033 mg/l had either increased or decreased CFU-E numbers and those in male offspring exposed in utero to 0.066 mg/l were increased. The numbers of granulocytic/macrophage colony forming cells were increased in the male and female offspring from the top-dosed group and decreased in the male offspring in the mid-dosed group.

**Source:** BP Chemicals Ltd LONDON  
25-OCT-2000

(607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 6-week-old offspring  
**Doses:** 5, 10, 20 ppm/day (converts to 0.017, 0.033, 0.066 mg/l/day); 5-10 mice/dose  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 6 weeks of age, one male and one female offspring were selected from each litter, sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells.

**Result:** CFU-E numbers were equivalent to the control values except in the males exposed to 0.033 mg/l in utero which displayed significant variations from the controls.

**Source:** BP Chemicals Ltd LONDON  
25-OCT-2000

(607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 12-week-old offspring  
**Doses:** 10 ppm/day (converts to 0.033 mg/l/day); 5-10 mice/dose  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 10 weeks of age, the offspring [numbers unspecified] were re-exposed for 2 weeks to 0.033 mg/l. After the 2 weeks, the animals were sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells. Control offspring exposed in utero to air were similarly exposed at 10 weeks of age to 0.033 mg/l benzene for 2 weeks.

**Result:** The in utero benzene-exposed offspring showed a marked reduction in the numbers of bone marrow CFU-E on re-exposure. No such effect was seen in the in utero air-exposed animals. Both groups of offspring had decreased splenic granulocyte/macrophage colony forming unit-culture numbers on re-exposure but this was more severe in the in utero benzene-exposed offspring.

**Source:** BP Chemicals Ltd LONDON  
25-OCT-2000

(607)

**Species:** mouse **Sex:** female  
**Strain:** other  
**Route of administration:** inhalation  
**Exposure period:** gestation days 5-14  
**Frequency of treatment:** daily/three 4-hour exposures per day  
**Duration of test:** gestation day 17  
**Doses:** 0.5, 1.0 mg/l; 15 mice/dose  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** CFLP (LATI-Gödöllő, Hungary) strain mice were used. Control group consisted of 115 females exposed to air. Dams were killed on day 17 and fetuses examined as described by Hudak A. & Ungvary G. Toxicology 11, 55-63, 1978.

**Result:** No effects were seen on the numbers of live or dead or resorbed fetuses or on the incidence of minor anomalies or total malformations. The incidences of weight and skeletal retarded fetuses were increased, this being statistically significant.

**Source:** BP Chemicals Ltd LONDON  
24-OCT-2000

(1188)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** The study was performed to investigate predominantly effects on the developing hematopoietic system consequently to prenatal benzene exposure. Thus, duration of the test is related to the different time points of investigations of offspring  
**Doses:** 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l); 5-10 mice/dose  
**Control Group:** yes, concurrent vehicle  
**Method:** other  
**GLP:** no data  
**Test substance:** other TS: glass distilled benzene of chromatographic quality  
**Remark:** Benzene chamber concentration measured by infrared analyser. Control group consisted of five to ten females exposed to filtered conditioned air. Exposed pregnant mice were either sacrificed, their uteri removed and the number of live, dead and resorbed fetuses recorded or were allowed to proceed through normal parturition and their offspring subjected to further investigations.  
  
At sacrifice on gestation day 16 two male and two female fetuses/litter were weighed and examined for any external gross morphological malformations and fetal livers were removed for enumeration of haematopoietic colony forming cells.  
  
Offspring at 2 days of age: two male and two female neonates/litter were selected, weighed and examined for any external gross morphological malformations. Their livers were removed for enumeration of hematopoietic colony forming cells.  
  
Offspring at 6 weeks of age: one male and one female were selected from each litter, sacrificed and their spleens and femurs removed for enumeration of hematopoietic colony forming cells.  
  
Offspring at 12 weeks of age: offspring exposed in utero to 10 ppm had been re-exposed to 10 ppm benzene at 10 weeks of age for a total of two weeks. At the age of 12 weeks these animals (numbers unspecified) were sacrificed and their spleens and femurs removed for enumeration of hematopoietic colony forming cells.  
**Result:** It is reported from the study that for all exposure concentrations of benzene employed, litter sizes and weights as well as the numbers of dead, resorbed and malformed fetuses were within the control limits.  
  
In fetuses at day 16 of gestation, changes in the numbers of both erythroid precursor cells (i.e. colony forming

unit-erythroid, CFU-E; and burst forming unit-erythroid, BFU-E) were observed, the more mature, CFU-E, cells being affected to a greater extent by all exposure concentrations. The number of foetal CFU-E cells were significantly increased at 0.017 (5 ppm) and 0.033 mg/l (10 ppm) and significantly decreased at 0.066 mg/l (20 ppm).

Neonates (offspring at two days of age): no differences in CFU-E numbers were noted between the low-dose (5 ppm) neonates and the controls. Neonates from in utero exposure to 0.033 mg/l (10 ppm) had either increased or decreased CFU-E numbers and those of the male neonates from in utero exposure to 0.066 mg/l (20 ppm) were increased. The numbers of granulocytic/macrophage colony forming cells were increased in male and female neonates from the top-dosed (20 ppm) group and decreased in male neonates from the mid-dosed (10 ppm) group.

Offspring at 6 weeks of age: CFU-E numbers were equivalent to their respective controls except in the males from in utero exposure to 0.033 mg/l (10 ppm) which displayed significant variations from their controls.

Offspring at 12 weeks of age: the in utero benzene-exposed offspring showed a marked reduction in the number of bone marrow CFU-E on re-exposure. No such effect was seen in the in utero air-exposed offspring. Both groups of offspring had also decreased splenic granulocyte/macrophage colony forming unit-culture numbers on re-exposure, however, the decrease was more severe in the in utero benzene-exposed offspring.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn;  
**Flag:** Risk Assessment  
17-JUN-2005 (608)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 2-day-old neonates  
**Doses:** 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l);  
5-10 mice/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. Two male and two female neonates/litter were selected at 2 days of age, weighed and examined for any external gross morphological malformations. Livers were removed for enumeration of haematopoietic colony forming cells. Benzene chamber concentration measured by infrared analyser.  
Reliability: 3 (not valid)

Significant methodological deficiencies predominantly effects on hematopoietic system studied (low number of animals, duration of experiment, only gross external malformations studied in 4 mice)

**Result:** No difference in CFU-E numbers were noted between the low-dose offspring and the controls. Offspring exposed, in utero, to 0.033 mg/l had either increased or decreased CFU-E numbers and those in male offspring exposed in utero to 0.066 mg/l were increased. The numbers of granulocytic/macrophage colony forming cells were increased in the male and female offspring from the top-dosed group and decreased in the male offspring in the mid-dosed group.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
25-OCT-2000 (607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 6-week-old offspring  
**Doses:** 5, 10, 20 ppm (converts to 0.017, 0.033, 0.066 mg/l);  
5-10 mice/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 6 weeks of age, one male and one female offspring were selected from each litter, sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells.  
Reliability: 3 (not valid)  
Significant methodological deficiencies only effects on hematopoietic system studied

**Result:** CFU-E numbers were equivalent to the control values except in the males exposed to 0.033 mg/l in utero which displayed significant variations from the controls.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
25-OCT-2000 (607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily/6 hours per day  
**Duration of test:** 12-week-old offspring  
**Doses:** 10 ppm (converts to 0.033 mg/l); 5-10 mice/dose  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of five to ten females exposed to filtered conditioned air. Exposed, pregnant mice were allowed to proceed through normal parturition. At 10 weeks of age, the offspring [numbers unspecified] were re-exposed for 2 weeks to 0.033 mg/l. After the 2 weeks, the animals were sacrificed and their spleens and femurs removed for enumeration of haematopoietic colony forming cells. Control offspring exposed in utero to air were similarly exposed at 10 weeks of age to 0.033 mg/l benzene for 2 weeks.  
Reliability: 3 (not valid)

**Result:** Significant methodological deficiencies only effects on hematopoietic system studied  
The in utero benzene-exposed offspring showed a marked reduction in the numbers of bone marrow CFU-E on re-exposure. No such effect was seen in the in utero air-exposed animals. Both groups of offspring had decreased splenic granulocyte/macrophage colony forming unit-culture numbers on re-exposure but this was more severe in the in utero benzene-exposed offspring.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
25-OCT-2000

(607)

**Species:** mouse **Sex:** female  
**Strain:** Swiss Webster  
**Route of administration:** inhalation  
**Exposure period:** g.d.5-16  
**Frequency of treatment:** 6h/day  
**Duration of test:** 10 consecutive days  
**Doses:** 10 ppm  
**Control Group:** no

**Result:** Fetuses from day 16 of gestation had been examined. From this experiment a significant reduction in the numbers of CFU-E from ( 10 ppm benzene exposed) fetal livers in the males is reported, whereas no changes at all were observed for the female fetuses. This latter finding is in contrast to the findings of the study of Keller and Snyder (1988), from which increases in the numbers of hepatic CFU-E in fetal offspring were observed with the identical treatment protocol.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
17-JUN-2005

(247)

**Species:** rabbit **Sex:** female  
**Strain:** New Zealand white  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-19  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 29  
**Doses:** 0.5, 1.0 mg/l; 11 and 15 rabbits respectively  
**Control Group:** yes, concurrent no treatment

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavédelem, Munka-és Uzemegészségügy 29, 207-216, 1983.

**Result:** The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and statistically significant increases in the number of dead or resorbed foetuses, mainly arising from abortion, and the percentage of minor anomalies. At 0.5 mg/l, slight non-significant decreases in maternal weight gain and increases in the numbers of dead or resorbed foetuses and minor anomalies was reported. The investigators did not consider benzene to be teratogenic.

**Source:** BP Chemicals Ltd LONDON  
24-OCT-2000

(1188)

**Species:** rabbit **Sex:** female  
**Strain:** New Zealand white  
**Route of administration:** inhalation  
**Exposure period:** gestation days 6-19  
**Frequency of treatment:** daily/24 hours per day  
**Duration of test:** gestation day 29  
**Doses:** 0.5, 1.0 mg/l; 11 and 15 rabbits respectively  
**Control Group:** yes, concurrent vehicle

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Control group consisted of 60 dams exposed to air. Dams were killed on day 29 and foetuses examined as described by Ungvary G. Munkavédelem 29, 207-216, 1983.

Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions  
no data about test substance, partly insufficient  
documentation of methods, 2 concentrations tested

**Result:** The top dose caused statistically significant decreases in maternal weight gain and mean foetal weight and statistically significant increases in the number of dead or resorbed foetuses, mainly arising from abortion, and the percentage of minor anomalies. At 0.5 mg/l, slight non-significant decreases in maternal weight gain and increases in the numbers of dead or resorbed foetuses and minor anomalies was reported. The investigators did not

consider benzene to be teratogenic.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
17-JUN-2005 (1188)

**Species:** other: mice and rabbits **Sex:** female  
**Strain:** other: CF-1 and New Zealand white  
**Route of administration:** inhalation  
**Exposure period:** mice: days 6 -15 of gestation  
rabbit: days 6 - 18 of gestation  
**Frequency of treatment:** 7 hours/day  
**Duration of test:** 18 - 29 days  
**Doses:** single dose level of 500 ppm  
**Control Group:** yes, concurrent vehicle  
**NOAEL Maternal Toxicity:** 500 ppm  
**NOAEL Teratogenicity:** 500 ppm

**Method:** other: nonguideline  
**GLP:** no data  
**Test substance:** no data

**Result:** mice:  
No significant effects on incidences of pregnancy, average number of live fetuses, absorptions per litter or on maternal body weight gain. Fetal growth retardation (significantly decreased mean fetal body weight) and significant increases in the occurrence of several minor skeletal variants including delayed ossification of sternebrae, skull bones and of unfused occipital bones of the skull were observed. No fetal malformations.  
rabbits:  
No malformations in fetuses and no significant effects on incidences of pregnancy, average number of live fetuses, or of absorptions per litter were observed.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn  
**Flag:** Risk Assessment  
17-JUN-2005 (804)

**Species:** other: mouse, rabbit **Sex:** female  
**Strain:** other: CFLP (mouse), New Zealand white (rabbit)  
**Route of administration:** inhalation  
**Exposure period:** mouse: gestation days 6-15, sacrifice at day 18;  
rabbit: gestation days 7-20, sacrifice at day 30;  
the day of finding a vaginal plug was taken the first  
day of gestation  
**Frequency of treatment:** mouse: for 24 h/day continuously or for 3x4 h/day  
intermittently  
rabbit: for 24 h/day continuously  
**Duration of test:** mouse: day of sacrifice day 18 of pregnancy  
rabbit: day of sacrifice day 30 of pregnancy  
**Doses:** mouse/rabbit: 0.5, 1.0 mg/l (converting to about 155  
and 310 ppm); 15 animals/dose  
**Control Group:** yes, concurrent vehicle  
**Method:** other  
**GLP:** no data  
**Test substance:** no data  
**Remark:** mouse: CFLP (LATI-Goedelloe, Hungary) strain mice were  
used.  
Control group consisted of 115 females exposed to air. Dams  
were killed on day 18 of pregnancy and fetuses examined as  
described elsewhere (Hudak A. & Ungvary G. Toxicology 11,  
55-63, 1978).  
rabbit: Control group consisted of 60 dams exposed to air.  
Dams were killed on day 30 of pregnancy and fetuses  
examined as described elsewhere (Ungvary G. Munkavedelem 29,  
207-216, 1983).  
**Result:** mouse:  
no effects were seen on the numbers of live fetuses or on  
the percentage of minor anomalies or of total malformations.  
A statistically significant increase of the ratio of weight  
retarded and of skeletally retarded fetuses as well as an  
increased ratio of absorbed or dead fetuses was observed at  
both concentration levels.  
rabbit:  
at 1.0 mg/l there were statistically significant decreases  
in maternal weight gain, mean fetal body weights and an  
increase in the number of abortions as well as increased  
ratios for dead or reorbed fetuses and for minor  
abnormalities. No evidence for developmental toxicity was  
observed at the lower dose level of 0.5 mg/l.  
**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn  
**Flag:** Risk Assessment  
17-JUN-2005 (1189)

**Species:** mouse **Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure period:** gestation days 12-15  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 1.0 ml/kg bw/day (converts to 880 mg/kg bw/day);  
group size unspecified  
**Control Group:** no data specified  
**NOAEL Maternal Toxicity:** = 880 mg/kg bw  
**NOAEL Teratogenicity:** = 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene administered in cotton seed oil.  
**Result:** No statistically significant benzene-related change was seen  
in the incidence of malformations.

**Source:** BP Chemicals Ltd LONDON  
25-OCT-2000

(814)

**Species:** mouse **Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure period:** gestation days 6-15  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880  
mg/kg bw/day); group sizes unspecified  
**Control Group:** yes, concurrent vehicle  
**NOAEL Maternal Toxicity:** = 880 mg/kg bw  
**NOAEL Teratogenicity:** = 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene administered in cotton seed oil.  
**Result:** No statistically significant benzene-related change was seen  
in the incidence of malformations.

**Source:** BP Chemicals Ltd LONDON  
25-OCT-2000

(814)

**Species:** mouse **Sex:** female  
**Strain:** NMRI  
**Route of administration:** gavage  
**Exposure period:** 6.- 15. Tag der Traechtigkeit  
**Frequency of treatment:** einmal taeglich  
**Duration of test:** bis zum 18. Tag der Traechtigkeit  
**Doses:** 563; 1688 mg/kg  
**Control Group:** yes, concurrent vehicle  
**NOAEL Teratogenicity:** 563 mg/kg bw

**Method:** other: nach den Richtlinien der FDA (1966)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Remark:** Reliability: 3 (ungueltig)  
entspricht nicht den heutigen Kriterien; Dosierungen hoeher als der Limit-Test; Maeuse weisen bei hoher Maternaltoxizitaet vermehrt Missbildungen auf

**Result:** Die Versuchs- und Kontrollgruppen bestanden aus je 19 bis 23 Muttertieren.  
Bereits in der niederen Dosisgruppe wurde maternale Toxizitaet in Form von verringerter Gewichtszunahme und, ab dem 11. Versuchstag, Unruhe, Zittern oder Apathie festgestellt. In der hohen Dosisgruppe traten diese Symptome bereits ab dem 8. Versuchstag auf. Ein Tier hatte einen Abort von 8 Feten.  
In der niederen Dosisgruppe wurden weder embryo- noch fetotoxischen Wirkungen festgestellt, auch traten keine vermehrten Missbildungen auf.  
Die hohe Dosis wirkte embryotoxisch, 23 % der Keime waren abgestorben (Kontrollgruppen 14 %). Das Gewicht der Feten war verringert. Die Feten wiesen vermehrt (bei 12/175 Feten) Missbildungen auf. (In einem Wurf wurde 6 mal verkuerzter Schwanz, sowie Spina bifida bei 3 Feten festgestellt, 1 Knickschwanz in einem anderen Wurf.)

**Source:** BASF AG Ludwigshafen  
13-DEC-1996

(78)

**Species:** mouse **Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure period:** gestation days 12-15  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 1.0 ml/kg bw/day (converts to 880 mg/kg bw/day);  
group size unspecified  
**Control Group:** no data specified  
**NOAEL Teratogenicity:** = 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Benzene administered in cotton seed oil.  
Reliability: 4 (validity cannot be judged)  
Abstract

**Result:** A significant increase in maternal lethality and embryonic  
resorption was seen. Foetal weight was significantly  
reduced.

No statistically significant benzene-related change was  
reported in the incidence of malformations.

**Source:** Deutsche Shell Chemie GmbH Eschborn

25-OCT-2000

(814)

**Species:** mouse **Sex:** female  
**Strain:** CD-1  
**Route of administration:** gavage  
**Exposure period:** days 6-15 of gestation with different dosages;  
days 12-15 of gestation with single dosage  
**Frequency of treatment:** daily  
**Duration of test:** no data  
**Doses:** 0.3, 0.5, 1.0 ml/kg bw/day (converts to 264, 440, 880  
mg/kg bw/day); group sizes unspecified  
**Control Group:** yes, concurrent vehicle  
**NOAEL Teratogenicity:** = 880 mg/kg bw

**Method:** other  
**GLP:** no data  
**Test substance:** no data

**Remark:** Data on this investigation are only available in form of an  
abstract.

Benzene was administered in cotton seed oil.

**Result:** For the dose levels of 0.5 and 1.0 ml/kg (days 6-15) a  
significant increase in maternal lethality and in embryonic  
resorption was reported. The same findings were reported to  
be obtained after shorter exposure (days 12-15) performed  
with the dosage of 1.0 ml/kg bw for which resorptions  
occurred later in gestation. Fetal weight was reported to be  
significantly reduced at all three doses. No statistically  
significant benzene-related change was reported in the  
incidence of malformations.

**Source:** German rapporteur; Deutsche Shell Chemie GmbH Eschborn;  
**Flag:** Risk Assessment

17-JUN-2005

(815)

**Species:** mouse **Sex:** female  
**Strain:** NMRI  
**Route of administration:** gavage  
**Exposure period:** 6.- 15. Tag der Traechtigkeit  
**Frequency of treatment:** einmal taeglich  
**Duration of test:** bis zum 18. Tag der Traechtigkeit  
**Doses:** 563; 1688 mg/kg  
**Control Group:** yes, concurrent vehicle  
**NOAEL Teratogenicity:** 563 mg/kg bw

**Method:** other: nach den Richtlinien der FDA (1966)  
**GLP:** no  
**Test substance:** as prescribed by 1.1 - 1.4

**Result:** Die Versuchs- und Kontrollgruppen bestanden aus je 19 bis 23 Muttertieren. Bereits in der niederen Dosisgruppe wurde maternale Toxizitaet in Form von verringerter Gewichtszunahme und, ab dem 11. Versuchstag, Unruhe, Zittern oder Apathie festgestellt. In der hohen Dosisgruppe traten diese Symptome bereits ab dem 8. Versuchstag auf. Ein Tier hatte einen Abort von 8 Feten. In der niederen Dosisgruppe wurden weder embryo- noch fetotoxischen Wirkungen festgestellt, auch traten keine vermehrten Missbildungen auf. Die hohe Dosis wirkte embryotoxisch, 23 % der Keime waren abgestorben (Kontrollgruppen 14 %). Das Gewicht der Feten war verringert. Die Feten wiesen vermehrt (bei 12/175 Feten) Missbildungen auf. (In einem Wurf wurde 6 mal verkuerzter Schwanz, sowie Spina bifida bei 3 Feten festgestellt, 1 Knickschwanz in einem anderen Wurf.)

**Source:** BASF AG Ludwigshafen  
**Reliability:** (3) invalid  
entspricht nicht den heutigen Kriterien; Dosierungen hoeher als der Limit-Test; Maeuse weisen bei hoher Maternaltoxizitaet vermehrt Missbildungen auf

08-NOV-1995

(78)

### **5.8.3 Toxicity to Reproduction, Other Studies**

**Type:** other: benzene and human pregnancy

**Method:** Subjects  
Eligible women were current employees of the Beijing Yanshan Petrochemical Corporation (BYPC) who had had a single birth at the BYPC staff hospital between May 1996 and December 1998. Those who had multiple births, births with major congenital defects or a medically diagnosed gynaecological or endocrine disorder were excluded from the study. Women exposed to other solvents, who smoke or drank alcohol were also excluded. The final sample of 792 women included 354 with benzene exposure and 438 not exposed to benzene.

#### Exposure

Exposure was assessed by (i) a walk through field study to identify the production processes, job titles and job tasks being conducted by women at the site; (ii) personal air monitoring (benzene, toluene, styrene, xylene) on a sub-set of 132 female workers on randomly-selected days, together with an industrial hygienist's assessment of each woman's exposure based upon a knowledge of her work duties; (iii) predicting the exposure characteristics of the remainder of the subjects based on a knowledge of their work duties together with use of a standardized algorithm developed from activity number (ii).

#### Other information

Information on exposure to reproductive hazards (eg chemical / physical hazards, smoking, work stress) other than solvents was collected by interview.

#### Assessment of birth outcomes

Birth weight was measured in the delivery room by a trained nurse, and was accurate to 10g. Adequate information on pregnancy history was available to permit calculation of gestational age in exact days (rather than complete weeks as is normally the case).

#### Statistics

Linear regression was used to examine the relationship between birth weight and major occupational and environmental exposures. Exposure variables were treated as binary or dummy variables. Perceived work stress and physical exertion were dichotomised because the highest category contained few subjects. All the multivariate models were adjusted for known or potentially important confounders. The final model showed a good fit with the residuals showing no significant departure from normal distribution ( $p > 0.10$ , Shapiro-Wilk test).

**Result:** Subjects  
The benzene-exposed population was considered "low risk", by the researchers, with most women at their optimal reproductive age, most being the ideal weight for height, non-smokers and non-alcohol drinkers.

**Exposure**

No detailed exposure data are presented. The full shift TWA exposure to benzene is stated as 0.017 ppm to 0.191 ppm, with an overall mean of 0.112 ppm and a median of 0.033 ppm. Styrene, toluene and xylene were stated to be below 1 ppm.

**Pregnancy parameters**

The overall mean(SD) birth weight for this group was 3427(441) g. The gestational age had a near normal distribution, with a mean(SD) of 39.9(1.4) weeks. The mean birth weight was 82 g lighter, and the mean gestational age 0.2 weeks shorter, for the group exposed to benzene in comparison to the controls.

When the data were adjusted for gestational age, benzene exposure (-58 g,  $p = 0.044$ ) and perceived work stress (-84 g,  $p = 0.026$ ) were associated with a significant reduction in birth weight. Exposure to noise and other hazards was without significant effect.

Multivariate analysis demonstrated a significant interaction between benzene exposure and workplace stress. Adjusted mean birth weight was 3445 g for the control, 3430 g for those with only exposure to benzene, 3426 g for those with only work stress, and 3262 g for those with exposure to both benzene and work stress. The group with both exposures showed a 183 g reduction in birth weight relative to the controls.

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors conclude that low level exposure to benzene and work stress interact to reduce birth weight.

25-APR-2002

(188)

**Type:** other: human menstrual cycle

**Method:****Subjects**

The study population comprised newly-wed women and nonparous married women aged 20 - 40 yr who worked for the Beijing Yanshan Petrochemical Corporation. 1408 women with occupational exposure to benzene, toluene, styrene and xylene (BTSX) were recruited to the study, which ran between 1994 and 1998.

**Exposure assessment**

Each woman's exposure to organic solvents was determined by an evaluation of the production/processing activity underway in her place of work. She was classified as exposed if a particular aromatic solvent (BTSX) was used in the vicinity where she worked, otherwise she was classified as unexposed. The number of years of exposure to a specific solvent was also assessed by questionnaire, as was potential exposure to other potential reproductive toxicants.

**Assessment of menstrual patterns**

Information on each subject's menstrual pattern in the year before enrollment into the study was assessed by

questionnaire, and included average cycle length, longest and shortest cycle length, average duration of bleeding, perceived irregularity, intermenstrual spotting and perimenstrual symptoms.

#### Statistical analysis

Multiple logistic regression was used to estimate the effect of exposure variables, with adjustment for co-variates such as passive smoking, rotating shift work, physical or mental stress etc.

#### Result:

##### Definitions:

Oligomenorrhea was defined as an average cycle length of >35 days;

Amenorrhea was defined as an average cycle length of >90 days

Polymenorrhoea as an average cycle <21days;

Prolonged bleeding duration or menorrhagia was defined as a period >7 days.

138 of the 1408 study participants (9.8%) had oligomenorrhea, and 6 of these were amenorrheic. A higher prevalence of oligomenorrhea was found among subjects exposed to 'any aromatic solvent' (12.7%) than among the unexposed subjects (8.5%). Women with older age, higher body mass index, passive smoking, perceived workplace stress or involved in lifting work had a higher percentage of long menstrual cycles.

Results from multivariate logistic regression analysis of oligomenorrhea showed that exposure to 'any aromatic solvent' was associated with an adjusted OR of 1.76 (CI 1.08 - 2.82) compared with the 'no exposure' group. A trend for increased frequency of oligomenorrhea was apparent for individual exposure to each of the 4 solvents with a 12.7% incidence with benzene (OR 1.34) versus 8.5% in controls. Styrene exposure showed the greatest increase (14.5% incidence, OR 1.65)

Duration of employment was longer in the exposed group (mean 4.9 yr) compared to the controls (4.2 yr). A 7% increase in the odds of oligomenorrhea was associated with each additional year of exposure to aromatic solvents, and 3 or more years exposure was associated with a 53% increase in oligomenorrhea. (OR 1.53, CI 1.00 - 2.34).

#### Source:

A.K. Mallett Surrey

#### Conclusion:

The authors conclude that occupational exposure to aromatic solvents is associated with a trend toward increased frequency of oligomenorrhea.

15-MAR-2002

(198)

**Type:** other: effects on pregnancy

**Method:** SUBJECTS  
The study population was recruited from female employees working for the Beijing Yanshan Petrochemical Corporation (BYPC), Peoples Republic of China. Eligible women were defined as non-drinking, non-smoking mothers who had a live single birth at the BYPC staff hospital between June 1995 and June 1997. This resulted in recruitment of 302 reference (non-exposed) individuals and 240 benzene-exposed subjects (total: 542). Clinical and personal data were collected by interviewer-administered questionnaire. In general, pregnant employees stopped working at 28 weeks of gestation.

#### GESTATIONAL AGE

The first date of the last menstrual period recorded at the first prenatal visit was used to estimate gestational age in days. The authors considered that prevailing social and administrative considerations within the Peoples Republic of China meant that this would be reliably noted by the participants.

#### EXPOSURE ASSESSMENT

The authors note that, in general, time-weighted average for benzene exposure in the BYPC facility was a range 0.017-0.191 ppm. Maternal occupational exposure to benzene was assessed qualitatively according to a woman's workshop, job title and job activity. Air sample measurements for benzene, toluene, styrene, xylene and their derivatives were obtained from major workshops during the study period. The subjects were then classified into 3 groups based upon (1) no exposure to organic solvents; (2) exposure to benzene; and (3) exposure to other solvents (including styrene, toluene, xylene and their derivatives but not benzene). Note: only results for the benzene-exposed and non-benzene-exposed individuals were presented in this study report.

#### GENOTYPING

##### POLYMORPHISM OF CYP1A1

Maternal genomic DNA was subject to 35 rounds of amplification using the polymerase chain reaction, followed by digestion of the products with HincII and agarose gel electrophoresis with ethidium bromide staining and visualisation under UV light. Homozygous wild-type individuals for cytochrome P450 1A1 (CYP1A1) show 139- and 32-base pair (BP) fragments (BPF), while heterozygous subjects show 4 bands at 139, 120, 32 and 19 BP, respectively. Homozygous rare-allele individuals show only 120-, 32- and 19 BP bands.

##### POLYMORPHISM OF GSTT1

Wild type homozygous glutathione S-transferase (GSTT1) was indicated by an amplified 457-BP product after PCR and agarose gel electrophoresis, while the null genotype was characterised by the absence of the 457-BP band.

## STATISTICAL METHODS

Multivariate analysis and multiple linear regression analysis were used to determine if there was any relationship between maternal allele frequency of CYP1A1, GSTT1 polymorphisms, maternal / infant characteristics and gestational age in the reference- and benzene exposed populations.

**Result:**

## GENERAL

This was an overall low-risk population, with the majority of women at their optimal reproductive age. Ninety-seven percent of the infants were born at term, with a mean gestational age of 39.9 wk. Women from the reference- and benzene exposed populations were similar in terms of CYP1A1 HincII- and GSTT1 allele frequency.

## GESTATIONAL AGE STRATIFIED BY CYP1A1 AND GSTT1

As estimated from multiple linear regression, benzene exposure was associated with a decrease in mean gestational age of -0.29 wk ( $P < 0.05$ ) in the total sample (that is, irrespective of maternal genotype for CYP1A1 and GSTT1).

Women with the AA genotype for CYP1A1 exhibited a -0.54 wk shortening ( $P < 0.001$ ) of gestational age when exposed to benzene than did women with the Aa/aa genotype (+0.06 wk, n/s), after adjustment for other covariates.

There was a negative non-significant association between benzene exposure and gestational age in both the presence (-0.28 wk) and absence (-0.24 wk) of the genotype for GSTT1.

In the absence of benzene exposure, none of the maternal genotypes were significantly associated with shortened gestational age, however in the presence of benzene exposure there was a significant association with the CYP1A1 AA-GSTT1 absent genotype (-0.79 wk;  $P < 0.002$ ) and CYP1A1 AA-GSTT1 present genotypes (-0.50 wk;  $P < 0.05$ ).

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The authors conclude that low level exposure to benzene is associated with shortened gestation. This association is modified by maternal genotype, in this case polymorphism for CYP1A1 and GSTT1.

25-JAN-2003

(1233)

**Type:** other: effects on sperm and early fetal loss

**Remark:** Chinese language article, translation unavailable:

ABSTRACT:

" Environmental monitoring and biological monitoring were conducted in 126 employees exposed to mixed benzene and 60 unexposed ones selected from a large petrochemical corporation and a large chemical fiber corporation. Quality of semen in 70 male workers in the exposed group and 90 male internal controls and 132 male external controls were analyzed, and urinary beta-human chorionic gonadotropin (beta-hCG) was determined for 42 female exposed workers and 49 female internal controls and 118 external controls.

Exposure to mixed benzene mainly existed in low concentrations (0.7-27.0 mg/m<sup>3</sup>) in the petrochemical corporation, and urine concentration of t,t-MA in the workers correlated significantly to benzene concentration in ambient air of the workshops (r = 0.64, P = 0.03).

2. The sperm progression and motility in the exposed (1.97 +/- 0.63 and 0.55 +/- 0.16) and the internal control (1.97 +/- 0.69 and 0.55 +/- 0.17) groups were significantly (P 0.05) lower than those in the external control group (2.56 +/- 0.61 and 0.60 +/- 0.13), however, the sperm abnormality rates in the exposed and internal control groups were significantly (P 0.01) higher than those in the external control group, and the summary abnormality rate of sperm in the exposed group (85.71%) was higher than that in the internal (70.00%) and external control (76.51%) groups (P 0.05).

3. The incidence rates of the VEFL in female workers and the cyclic occurrence of VEFL both in the exposed and internal control groups (10.42% and 9.60%) were higher than those in the external control group (5.09%), with statistical significance (P 0.05)."

**Source:** A.K. Mallett Surrey

**Conclusion:** Exposure to low concentrations of mixed benzene may be correlated with decreased quality of semen and VEFL in occupational workers, and urinary t,t-MA and beta-hCG can be used as biomarkers of exposure and effect, respectively.

05-MAY-2003

(1234)

**Type:** other: effects on female reproductive hormones

**Method:** SUBJECTS  
The study population comprised 50 female workers (mean age 28.1 yr, mean period of employment 7.3 yr) from an undefined petrochemicals facility in the Peoples Republic of China, with potential exposure to benzene, toluene and xylene (isomer undefined). An "internal control group" was recruited from the locale of the plant (35 subjects, mean age 27.9 yr) while an "external control group" (35 subjects) was recruited from the vicinity of a plant manufacturing chemical fibres. The selection criteria included avoidance of oral contraceptives for over 6 mo, greater than 1 yr since having an abortion, no more than 1 abortion or miscarriage and no history of reproductive disease.

#### EXPOSURE ASSESSMENT

Air was collected from the breathing zone using a 100 ml syringe, which was sealed and subsequently analysed using GC for benzene, toluene and xylene (no information on timing or frequency). Ambient exposure data were collected in the morning, afternoon and evening on 3 consecutive days.

#### ANALYSIS OF HORMONE LEVELS

A 5 ml early morning urine sample was collected from each subject and stored at -20 degrees C (no further details of number or collection frequency or storage). Samples were analysed by GC for FSH, pregnanediol-3-glucuronide and (undefined) estrone conjugate.

#### MENSTRUAL CYCLE CHARACTERISTICS

Each subject maintained a daily record of menstrual cycle details (no further information).

#### STATISTICAL METHODS

The data were analysed using one way ANOVA, Newman-Keuls test and Chi Square test. Hormone levels are presented as the geometric mean.

**Remark:** Reviewer's note: Original paper in Chinese, information obtained from translation, some study details unclear.

The authors conclude that exposure to low concentrations of benzene, toluene and xylene can interfere with normal levels of FSH, oestrogens and progesterones in the hypothalamic-pituitary-ovarian axis in female workers. However there are significant shortcomings in the exposure assessment underpinning this conclusion, and only graphical data (with no statistical indicators) are presented in the results. These may limit the overall reliability of the paper.

It is also stated that around one-fifth of the exposure measurements were 20-fold greater than the prevailing Chinese national standard of 40 mg/m<sup>3</sup> (13 ppm) ie a significant number of subjects were exposed to whole-shift benzene concentrations of 800 mg/m<sup>3</sup> (260 ppm). More

generalised effects would be anticipated under such conditions suggesting that an alteration in hormone levels in these individuals may well be secondary to systemic toxicity.

**Result:**

## EXPOSURE

Benzene, toluene and xylene were present in 29.1%, 7.6% and 2.7% of the ambient air samples, respectively. Air concentrations were 8.9 mg/m<sup>3</sup>, 2.9 mg/m<sup>3</sup> and 4.3 mg/m<sup>3</sup> for benzene, toluene and xylene, respectively (unclear if this refers to ambient or personal monitoring), and >64% of the samples contained <5 mg/m<sup>3</sup>. No benzene, toluene or xylene was detected in the internal or external control environments.

## MENSTRUAL CHARACTERISTICS

The length of the luteal phase was significantly shorter in the exposed population (13.7 d) when compared to the two reference groups (14.5d for internal controls; 15.2 d for external controls; basis for this conclusion not reported). Comment: it is noted that the difference between the exposed group and the internal control group (0.8 d) is highly comparable to the difference between the internal and external control groups (0.7 d) suggesting that factors other than benzene may have been involved. There were no significant differences in cycle length or incidence of menstrual abnormalities between the groups.

## REPRODUCTIVE HORMONES

FSH levels in the early follicular phase (14th and 14th day before ovulation) in the exposure group were significantly lower than in the external control groups (relationship to internal control group not stated). The concentration of pregnanediol-3-glucuronide in urine was significantly lower in the exposed subjects than in the internal and external control groups during the early follicular (on days 8-15 prior to ovulation) and luteal phases (on days 8-13 post-ovulation). The concentration of the estrone conjugate in urine from the exposed group was lower in the early follicular phase (12-15 days prior to ovulation) and before ovulation (3-6 days prior to ovulation) than in the controls. (No quantitative data available).

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The authors suggest that occupational exposure to low levels (<5 mg/m<sup>3</sup>) benzene, toluene and xylene was associated with a significant decrease in urinary FSH and other selected oestrogen metabolites in female workers, with a significant increase in length of the luteal phase of menstrual cycle. However considerable between-group variability was present in luteal phase values from the two non-exposed control populations suggesting that this difference was of doubtful biological significance. Occupational exposure measurements underpinning the study also appear to provide little evidence of a clear causal association with benzene exposure.

23-MAY-2003

(190)

**Type:** other: epidemiological data

**Result:** In this study, 30 women with symptoms of benzene toxicity were examined. The women worked in an environment containing benzene, however, the levels of benzene in air were not specified, but were assumed to have been much greater than 1 ppm. Twelve of these women had menstrual disorders (profuse or scanty blood flow and dysmenorrhea). Leukopenia was reported in four women, and in the majority of the women the neutrophils and platelets were also reduced. Ten of the 12 women were married. Of these 10 women, 2 had spontaneous abortions, and no births occurred during their employment even though no contraceptive measures had been taken. This led the investigators to suggest that benzene has a detrimental effect on fertility at high levels of exposure. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological examinations revealed that the scanty menstruations of five of the patients were due to ovarian atrophy. This study is limited in that an appropriate comparison population was not identified. Additionally, little follow-up was conducted on the 30 women with regard to their continued work history and possible symptoms of benzene toxicity.

**Source:** German rapporteur  
17-JUN-2005 (1206)

**Type:** other: epidemiological data

**Result:** In this study disturbances of the menstrual cycle were found in female workers exposed to aromatic hydrocarbons (benzene, toluene, xylene). The exposure levels of benzene and toluene were below 0.25 ppm. The observed group consisted of 500 women, 20-40 years old. One hundred controls were included in the study. The results showed that 21% of exposed women whose work was involved in sitting or standing had irregular menstrual cycles compared to 12% in the control group. Brief (up to 2), long (6-9), and prolonged (over 9 days) menstrual cycles were present in 26% of women who performed lifting during their work as compared to 13% in the control group. Irregular amounts of menstrual flow and pain were also observed in female workers exposed to aromatic hydrocarbons. The major limitations of this study are that the exposure occurred from a mixture of chemicals, level of exposure were not well defined, duration of exposure was not stated, and activities of the controls were not provided.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
17-JUN-2005 (773)

**Type:** other:epidemiological data

**Result:** This study examined the reproductive function and incidence of gynecological effects in 360 female gluing operators exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons both dermally and by inhalation. However, dermal exposure was considered to be negligible. The concentrations of benzene in the air were not well documented. When compared to female workers with no chemical exposure, there was no significant difference in fertility. However, female gluers had developed functional disturbances of the menstrual cycle. Additionally, as chemical exposure time increased, there were increases in the number of premature interruptions of pregnancy, the percentage of cases in which the membranes ruptured late, and the of cases of intrauterine asphyxia of the fetus. The study limitations (including lack of exposure history, simultaneous exposure to other substances, and lack of follow-up) make it difficult to assess the effects of benzene on reproduction.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(801)

**Type:** other: epidemiological data

**Result:** In the study conducted in the Love Canal area (New York, USA) by Heath, the outcome of pregnancy was evaluated in populations living in the proximity of waste sites where within a total of at least 248 chemicals also benzene had been identified. No clear increase in occurrence of spontaneous abortion, birth defects, or low infant birth weight was observed in women living next to the canal. The study limitations of inadequate sample size and lack of exposure history preclude an assessment of significance of these findings.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(497)

**Type:** other:epidemiological data

**Result:** In another study on the Love canal area, birth weight was assessed in 239 infants exposed during gestation life. There was an association between low birth weight (<2500 gms) and hazardous waste exposure. However, there were inherent problems in the study design and methodology. One of the study groups was comprised of low-income renters and predominantly black individuals. The methods also failed to include 235 families that were evacuated from Love Canal area.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(422)

**Type:** other:epidemiological data

**Result:** In this study birth weight was investigated in a cohort of chinese petrochemical workers in a plant that had been exposed to solvents including benzene, toluene, styrol and xylene. The cohort consisted of 354 benzene-exposed and 438 non-exposed participants. As an orientation for benzene during the shift in that plant a time weighted average (TWA) of 0.017 ppm (rubber plant) to 0.191 ppm (chemical plant No.1) was indicated. In this study in the exposed group any exposure to benzene was rated as a concentration detected for any of the 4 measured chemicals (benzene, toluene, styrol and xylene) by personal air sampling, which had been performed for 132 workers only, or by job title or by workshop. By using this definition reduced birth weight was associated with exposure to benzene as well as with perceived work stress. The adjusted mean birth weight was 3445 g (95% CI 3402 to 3489) among those without exposure to benzene and work stress, 3430 g (95% CI 3382 to 3477) for those with only exposure to benzene, 3426 (95% CI 3340 to 3513) for those with only work stress, and 3262 (95% CI 3156 to 3369) for those with exposure to both benzene and work stress. For this study an interaction was observed between exposure to benzene and percieved work stress.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(189)

**Type:** other: epidemiological data

**Result:** Effects of benzene exposure to menstrual disturbances had been investigated in an exploratory, cross-sectional retrospective study (Thurston et al., 2000). Based on a survey administered to about 3 000 workers of a chinese petrochemical company, 333 women had been identified with abnormal (>35 days or <21 days) menstrual cycle length (AMCL). Chemical exposure of study participants was assessed by using a questionnaire and coded by years according to the jobs for which exposure to a particular chemical had been reported. Among different variables, that had been explored during the study, longer exposures to benzene (several years) predicted probability of having AMCL. The adjusted odds of AMCL did not change significantly during the first 7 years of benzene exposure, however, the adjusted odds of having AMCL for each additional 5 years of benzene exposure was 1.71 (95% CI 1.27-2.31). Feeling stressed at work was also an important predictor for AMCL.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(1132)

**Type:** other: epidemiological data

**Result:** No statistically significant clusters of birth defects were found in populations living around the Drake Superfund site (Pennsylvania, USA), an area contaminated with benzene and other carcinogens (Budnick et al. 1984). However, the significance of this finding cannot be determined because of design methodology inadequacies including inadequate sample size and lack of quantification of exposure levels.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(168)

**Type:** other: epidemiological data

**Result:** In a study of subjects with known benzene exposure Forni et al. (1971) reported the case of a pregnant worker exposed to benzene in the air throughout the entire pregnancy. Although the woman had severe pancytopenia and an increased frequency of chromosomal aberrations, a healthy boy was delivered with no evidence of developmental effects and with no evidence of chromosomal alterations. In the following year a healthy girl was delivered.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(367)

**Type:** other: epidemiological data

**Result:** Increased frequencies of chromosome breaks and of sister chromatid exchange were found in lymphocytes from 14 children of female workers exposed by inhalation to benzene and other organic solvents (doses not specified) during pregnancy (Funes-Cravioto et al. 1977). No mention was made for the reasons of this investigation nor of whether the mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

**Source:** German rapporteur

**Flag:** Risk Assessment

17-JUN-2005

(383)

**Type:** other: transplacental transfer

**Result:** One toxicokinetic study showed that benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976).

**Source:** German rapporteur

17-JUN-2005

(309)

## 5.9 Specific Investigations

**Endpoint:** Hematotoxicity

**Method:** An expanded version of this study was reported subsequently by Qu et al. (2003) Validation and evaluation of biomarkers in workers exposed to benzene in China. Research Report 115, Health Effects Institute, Boston MA (see accompanying record).

### SUBJECTS AND METHODS

One hundred and thirty subjects were recruited from three factories in Tianjin province, China, where benzene was used as solvent (glue manufacture and use). Two nearby factories (food processing, flour production) without benzene exposure were used to recruit 51 control subjects (matched for age, gender and smoking habits). Inclusion criteria included at least 3 years employment with no known disease (as assessed by interview and tests of liver function and urine analysis). The study was conducted in 2 phases in spring 1997 (25 exposed + 25 unexposed) and spring 1998 (26 unexposed + 105 exposed).

An additional group of 11 workers was used to characterise formation and clearance of exposure markers over a weekend, while another 11 workers were used to assess intra- and inter individual variation in biomarker levels in samples collected on 3 consecutive Mondays.

### EXPOSURE ESTIMATES

Personal benzene exposure was assessed on 3-4 occasions over a one month period using passive monitoring (3M organic vapour monitors). Samples were analysed (NIOSH standard method) using GC.

### BLOOD CELL COUNTS

Samples of peripheral blood were stored (4 degrees C, up to 3 hr) prior to analysis for RBC, WBC and platelets (automated counter) and differential white cell counts (neutrophils, lymphocytes, eosinophils from a blood smear).

### URINARY ANALYSES

S-phenylmercapturic acid (S-PMA) and t,t-muconic acid (t,t-MA) was quantified by simultaneous liquid chromatography-electrospray tandem mass spectrometry. Albumin adducts of benzene oxide (BO-Alb) and 1,4-benzoquinone (1,4BQ-Alb) were also determined (Waidyanatha et al (1998) Chem Biol Interact 115, 117). Cotinine (indicator of smoking status) was determined by radioimmunoassay and creatinine using a Kodak Ektachem analyser.

### STATISTICAL METHODS

One-way ANOVA was used to compare multiple ordered exposure groups. Multiple regression analysis was used to assess the potentially confounding effect of sex, age, cotinine levels

**Result:** and toluene exposure on blood cell parameters.  
EXPOSURE TO BENZENE, TOLUENE AND XYLENES  
Benzene exposure was calculated and presented as the current daily exposure (based on biological monitoring), a 4-wk average exposure levels (based on monitoring data and biological monitoring) and the lifetime cumulative exposure (based on job histories and historical exposure data for the factory). Daily benzene levels for the exposed subjects were in a range 0.06-122 ppm with a median of 3.2 ppm. The 4-wk mean range was 0.08-54.4 ppm, and the cumulative lifetime exposure was 6.1-623.2 ppm-years.

#### HAEMATOLOGICAL CHANGES

Graphical data indicate that there were significant exposure-related decreases in RBC, WBC and neutrophils (all  $P < 0.001$ ) but no clear effect on lymphocytes.

Regression analysis revealed significant negative associations between benzene exposure and RBC ( $P < 0.05$ ), WBC ( $P < 0.001$ ) and neutrophils ( $P < 0.001$ ). In addition, after adjustment for potential confounders (sex, age, toluene, cotinine), there were also weak negative associations between benzene exposure and decreased lymphocyte ( $P < 0.01$ ) and monocyte counts (data not reported).

Analyses were performed to examine blood cell counts at current low levels of exposure (4-wk average exposure of 0.25 ppm benzene or lower). This showed statistically significant decreases in RBC, WBC and neutrophils in the exposed group, which remained after adjustment for confounding factors.

Comment: While this appears to be one of the key findings from this study, the underlying data were not obvious from tables and figures included in the publication. The reliability of the exposure estimates is also unclear since it is noted elsewhere (Qu et al., 2003) that changes in the low exposure workers may have been due to past cumulative exposure to benzene.

Graphical results demonstrated statistically significant decreases in RBC ( $P < 0.001$ ), WBC ( $P < 0.001$ ) and neutrophils ( $P < 0.001$ ) when blood cell counts were analysed relative to cumulative benzene exposure. (The authors comment that the differences RBC appeared primarily due to a difference between the exposed and unexposed population with little gradation in response to cumulative exposure, reducing confidence in the association.)

After adjustment for possible confounders, regression analyses of blood cell parameters relative to cumulative exposure showed strong inverse associations with RBC ( $P < 0.01$ ), WBC ( $P < 0.05$ ), neutrophils ( $P < 0.01$ ) and monocytes (not reported), with weaker associations for lymphocytes ( $P < 0.001$ ) and eosinophils (not reported).

An analysis of the relative contribution of benzene exposure duration and intensity showed that RBC ( $P < 0.01$ ; approx. 17%

decrease with 18 yr or more exposure) and neutrophils (P<0.001; approx. 21% decrease) were influenced by duration of exposure while RBC (P<0.01; approx. 15% decrease at 40 ppm-yr and above), WBC (P<0.01; 29% decrease), neutrophils (P<0.01; 38% decrease) and eosinophils (not reported) were affected by average exposure concentration per year.

An exposure-response regression analysis showed that benzene exposure intensity predicted decreases in RBC, WBC, lymphocyte, neutrophil, monocyte and eosinophil counts while exposure duration showed only a weak association with neutrophils. Hence benzene exposure intensity appeared more predictive of bone marrow depression than duration of exposure.

CORRELATION BETWEEN MARKERS OF EXPOSURE AND BLOOD CELL CHANGES

Significant correlation was found between the presence of S-PMA, t,t-MA, BO-Alb and 1,4BQ-Alb in urine and decreased RBC, WBC and neutrophil counts.

**Source:**  
**Conclusion:**

A.K. Mallett Surrey  
 Exposure-dependent decreases in red blood cell, white blood cell and neutrophil counts were reported in this study, with significant effects apparently present in subjects currently exposed to 0.25 ppm benzene or below (although confounding due to higher cumulative exposures in the past could not be excluded). Decreases in blood cell parameters correlated with the presence (but not level) of biomarkers of benzene exposure present in urine.

06-JUL-2005

(915) (916)

**Endpoint:** Hematotoxicity

**Method:** This report contains expanded details of some of the information contained in the preceding record by Qu et al. (2002) Hematological changes among Chinese workers with a broad range of benzene exposures. Am J Ind Med. 42, 275-285.

**Result:** BLOOD CELL COUNTS AND DIFERENTIATION  
 Both 4 wk mean benzene exposure levels and lifetime cumulative exposure levels were used when analysing any association between benzene exposure and blood cell parameters.

When analysed relative to 4 wk mean exposures, graphical data indicate that there were significant exposure-related decreases in RBCs (P<0.001), WBCs (P<0.001) and neutrophils (P<0.001) but no clear effect on lymphocytes or platelets.

Comparison of results for unexposed subjects with those from a sub-set of exposed subjects with 4 wk mean benzene exposures of 0.5 ppm or lower revealed statistically significant decreases in RBCs, WBCs and neutrophils after controlling for sex, age, smoking habits etc (lymphocytes unaffected):

Unexposed	Exposed
-----------	---------

No. subjects	51	16	
Female (%)	53	100	
Smoker (%)	31	0	
Age (yr)	33	36	
4 wk mean benzene (ppm)	0.004	0.14	
RBC (x 10 <sup>10</sup> /l)	463	393	(P = 0.0006)
WBC (x 10 <sup>6</sup> /l)	6671	5700	(P = 0.02)
Neutraphils (x 10 <sup>6</sup> /l)	4006	3254	(P = 0.02)

When blood cell counts were analysed relative to lifetime cumulative benzene exposure, RBCs (P < 0.001), WBCs (P < 0.001) and neutraphils (P < 0.0001) again decreased significantly in relation to increasing cumulative benzene exposure. The effect on RBC appeared to be primarily a difference between the unexposed and exposed groups (little gradation according to cumulative exposure, reducing confidence in the association). The approximate magnitude of these changes, obtained by interpolation from graphical data presented in the report presented as percentage of the unexposed controls, was as follows:

Benzene (ppm-yr)	RBC	WBC	Neutraphils
0	-----100%-----		
<30	88	102	93
<50	83	91	80
<100	88	93	83
100 and above	85	84	78

Since the 4 wk mean benzene concentration may not be the most appropriate measure for correlation with changes in cell counts, the authors also performed analyses using benzene exposure duration (no. years of exposure; median = 14 yr) and exposure intensity (estimated mean exposure concentration per yr; median = 2.7 ppm/yr). This showed that RBC and neutraphil values were also decreased significantly when analyses were based on exposure duration or exposure intensity. WBC was also decreased in the latter instance.

Comment: Other data, not included with the study report, apparently show that lymphocyte values were inversely associated with benzene exposure (both 4 wk mean concentration and cumulative exposure intensity) after adjustment for confounders.

**Source:**  
**Conclusion:**

A.K. Mallett Surrey  
 Significant decreases in red blood cells, white blood cells and neutraphils (and possible lymphocytes) were observed in human subjects occupationally exposed to benzene. The authors suggest that depression of multiple blood cell types is consistent with an effect of benzene on pluripotent stem cells.

30-MAR-2004

(915) (916)

**Endpoint:** Immunotoxicity

**Method:** Subjects  
The study group comprised 49 female workers, age 23-53 yr (mean 38 yr), who had been exposed to solvent mixtures for 1-33 yr (mean 17 yr). The controls were 26 healthy women, age 29-46 yr (mean 38 yr) employed in the confectionary industry. Based on the affiliation of the authors, it is presumed the study was carried out in Croatia.

Exposure measurements

The concentration of benzene and toluene in the the workplace was "measured continuously during the work shift in the middle of the working week" at 10 fixed sampling locations. Samples were collected onto charcoal-filled tubes (type not specified), desorbed with carbon disulfide and analysed by GC-FID. The exposure assessment was performed "blind" with respect to the immunological assays. The concentration of benzene and toluene in pre-shift blood (head space GC) and phenol (GC) in pre- and post-shift urine were also determined.

Immunological parameters

IgA, IgM and IgG in serum were determined by radial immunodiffusion, and serum IgE by radioimmunoassay. T- and B-lymphocytes were also quantified (method not specified).

Statistics

Student's t-test was applied to the immunological data, and the Wilcoxon test to the analyses of toluene and benzene in blood and urine. Spearman's rank correlation and multiple linear regression were used as appropriate.

**Result:** Exposure  
General air sampling in the 10 production areas showed that benzene levels were in a range 1.9 - 14.8 ppm (median 5.9 ppm), and toluene in a range 11.4 - 49.9 ppm (median 24.9 ppm). Benzene and toluene were present in pre-shift blood from shoe workers but undetectable in controls. Pre-shift urinary phenol was similar in both controls and workers, whereas post-shift urinary phenol was approx. 2-fold greater in shoe workers.

Immunological parameters

IgA, IgM, IgG and IgE levels in serum and the proportion of T-lymphocytes were indistinguishable between the two groups. The proportion of B-lymphocytes was decreased (approx 15%) in the shoe workers relative to the controls. The only parameter which showed a correlation with workplace benzene levels was serum IgG levels, which showed a slight positive trend (0.3115,  $p < 0.05$ ).

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors conclude that workplace exposure to benzene at levels of 15 ppm or below decreases the proportion of B-lymphocytes in the circulation. It should be noted, however, that there was no correlation between the various measures of benzene exposure included in the study and

B-lymphocyte numbers (correlation coefficients: -0.0033, -0.1619, 0.0740 and -0.1463 for external benzene concentration, pre-shift blood benzene, pre-shift- and post-shift urinary phenol respectively).

25-APR-2002

(126)

**Endpoint:** other: biomarkers and molecular epidemiology

**Method:** Subjects  
44 healthy workers (Shanghai, China) with current exposure to benzene and minimal exposure to toluene and other aromatic solvents. The same number of healthy controls without current or previous occupational exposure to benzene were enrolled from factories in the same area.

Exposure assessment  
Individual exposure was determined using passive organic vapor dosimetry badges on 5 separate days. Historic exposure estimates were used to calculate cumulative exposure. The assessments were performed "blind" with respect to the biomarker phases of the study. The concentration of benzene metabolites in urine was used to "confirm" the air monitoring data (methods not given).

Biomarkers of exposure  
Benzene oxide-hemoglobin (BO-Hb) and benzene oxide-albumin (BO-Alb) were quantified in blood samples from 43 exposed workers and 42 unexposed controls using GC-MS.

Biomarkers of effect  
Fluorescence in situ hybridization (FISH) was used to detect specific chromosomal aberrations in exposed individuals and controls. This included acute myelogenous leukemia (AML) specific aberrations.

**Remark:** Discussion paper describing methods and preliminary results from a molecular epidemiology study on Chinese workers.

**Result:** Exposure  
The median concentration of benzene in air was 31 ppm 8 hr TWA (range: 1 - 328 ppm). There was a strong positive correlation between urinary metabolite levels and air benzene levels (data not reported).

Biomarkers of exposure  
The median concentration of BO-Hb in controls (n=42), workers exposed <31 ppm (n=21) and workers exposed >31ppm (n=22) was 32.0, 46.7 and 129 pmol/g globin, respectively ( $r^2 = 0.67$ ,  $p < 0.0001$ ). The median concentration of BO-Alb in these 3 groups was 103 (n=19), 351 (n=7) and 2010 (n=12) pmol/g Alb, respectively ( $r^2 = 0.90$ ,  $p < 0.0001$ ).

Benzene exposure was associated with significant increases in hyperploidy of chromosome 8 and 21, as assessed using FISH ( $p$  trend  $< 0.001$  for controls, workers exposed <31ppm and workers exposed >31 ppm). The incidence of hyperploidy in the high-dose group was approximately double that of the controls. Benzene also increased the the rates of monosomy 5

and 7 but not monosomy 1, with increases in trisomy and tetrasomy frequencies of all three chromosomes. The authors conclude that FISH can detect benzene-induced, leukemia-specific chromosomal aberrations.

**Source:**  
12-SEP-2001

A.K. Mallett Surrey

(1060)

**Endpoint:**

Hematotoxicity

**Remark:**

A hypothesis generating review article. The authors speculate that bone marrow stromal cells become hyperresponsive to inflammatory mediators and growth factors after benzene administration, leading to over-production of nitric oxide and toxicity.

Following benzene treatment, mice show a marked enhancement of nitric oxide production in bone marrow cells stimulated with interferon gamma (IFG) and bacterial lipopolysaccharide (BLS). The sensitivity of bone marrow cells to the growth promoting effects of granulocyte-macrophage colony stimulating factor (GMCSF) and macrophage colony stimulating factor (MCSF) was also observed. The magnitude of the response was greater than that seen in mouse bone marrow cells after treatment IFG, BLS, GMCSF or MCSF in the absence of benzene pretreatment.

The authors suggest that benzene primes bone marrow cells for increased responsiveness to agents that induce nitric oxide. This appears to be due, at least in part, to an increased expression of nitric oxide synthetase mRNA. Ng-monomethyl-L-arginine, a nitric oxide synthase inhibitor, potentiated both the decrease in bone marrow cellularity and increase in nitric oxide production caused by benzene.

Treatment of mice with benzene metabolites (hydroquinone, 1,2,4-benzenetriol, p-benzoquinone) was found to sensitize bone marrow leukocytes to produce increased amounts of nitric oxide in response to IFG and BLS. These metabolites also increased the responsiveness of bone marrow cells to MCSF and GMCSF.

**Source:**  
12-SEP-2001

A.K. Mallett Surrey

(667)

**Endpoint:** Hematotoxicity

**Method:** A group of 30 CD1 male mice (age 8-12 wk, 10 mice/group) were given benzene (2 ml/kg) in corn oil by subcutaneous injection five times per week (Mon-Fri; Protocol 1) until a cumulative total of 10, 15 or 20 doses had been administered. A second group were treated three time per week (Mon, Wed, Fri; Protocol 2) until the same cumulative treatment schedule had been achieved. Control animals (two groups of 30 mice) received corn oil.

Two days after the final treatment, the animals were anaesthetised and exsanguinated by cardiac puncture. Haematological parameters (red cell count, white cell count, platelets, differential leukocyte- and reticulocyte count) were assessed using conventional methods. Total nucleated femoral bone marrow cell counts were recorded, and sections of spleen prepared for assessment by light microscopy.

Statistical comparison of results from the various groups was made using ANOVA and LSD Fisher tests.

**Result:**

Protocol 1

Treated animals exhibited irritability, lethargy poor condition and weight loss (42% reduction at study end). Internal organs appeared normal at necropsy, with the exception of the spleen which showed a >60% reduction in weight with extensive necrosis visible macroscopically. Haemoglobin content and erythrocyte-, leukocyte- and bone marrow cell counts were decreased 37%, 34%, 80% and 50%, respectively, at the end of the study. Microscopic examination revealed extensive necrosis of the spleen (affecting about 50% of the organ).

Protocol 2

Behavioural and clinical signs were similar to those noted in Protocol 1, however weight loss was less pronounced (12% reduction at study end). Spleen weights were 48% lower than control at the end of the study, but no macroscopic abnormalities were visible. Haemoglobin content and erythrocyte-, leukocyte- and bone marrow cell counts were decreased 12%, 48%, 62% and 62%, respectively, at the end of the study. Spleen tissue showed some micriscopic changes, with necrosis limited to foci in the germinal centre.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The authors conclude that their experimental approach offers a reproducible and inexpensive means for the induction of aplastic anemia in the mouse. However the observation of concurrent, severe behavioural, clinical and systemic changes suggests that these models may be of limited relevance to benzene-related blood diseases in humans.

26-FEB-2002

(684)

**Endpoint:** other: myelodysplastic syndrome

**Remark:** This case report centres on a 45 yr old man with myelodysplastic syndrome who started work at a petrochemical plant in Taiwan in 1977 where his duties involved charging a chemical reactor with benzene.

Routine haematological parameters were collected each year revealed anemia, leukocytopenia and thrombocytopenia since May 1994. He was referred for hospital investigation in 1996. Analysis of bone marrow aspirates showed he had myelodysplastic syndrome.

The patient was discharged from hospital and stopped working with benzene. Pancytopenia, weakness, fatigue dizziness, palpitation and excessive bleeding persisted.

Although the authors note that other substances have the potential to cause bone marrow toxicity, no information on potentially confounding co-exposures is presented in the paper. Apart from presenting longitudinal haematological data from one subject, it contains little information helpful to risk assessment.

**Source:**  
18-MAR-2002

A.K. Mallett Surrey

(193)

**Endpoint:** other: glutathione transferase activity in "benzene-induced leukemia" patients.

**Remark:** Chinese language article, translation unavailable:

Although the title refers to patients with "benzene-induced leukemia", the abstract provides no details on the causal basis of this diagnosis.

"Serum glutathione S-transferases (GSTase) activity was determined in benzene-induced leukemia patients, leukemia patients and control subjects by KMSL method. GSTase activity in benzene-induced leukemia patients was markedly lower than that in leukemia patients and healthy controls. GSTase activity in benzene-induced leukemia was significantly lower than that in leukemia group and control group, and GSTase activity in leukemia group was lower than that of the controls. GST.mu.gene deletion is an important factor affecting GSTase activity. The results show that the change in GSTase activity was an important factor in leukemia induction by benzene exposure, and GST.mu.gene deletion may be an important factor affecting the GSTase activities."

**Source:**  
04-MAR-2003

A.K. Mallett Surrey

(685)

**Endpoint:** other: metabolic polymorphisms and urinary biomarkers

**Method:**

SUBJECTS AND METHODS

169 police officers from municipal Rome were recruited for the study. 118 worked outdoors (traffic control duties; 88 male, 30 female) while 51 worked in doors (34 male, 17 female).

BIOMONITORING OF BENZENE EXPOSURE

Personal exposure to environmental benzene during the workshift (0700-1400) was determined using passive dosimeters. End-shift blood and urine samples were collected and analysed for benzene (GC method) and trans,trans muconic acid (HPLC) and S-phenylmercapturic acid (HPLC), respectively.

GENOTYPIC ANALYSES

DNA, isolated from heparinised peripheral blood, was analysed for polymorphisms in cytochrome P-4502E1 (CYP2E1) and NAD(P)H-quinone oxidoreductase (NQO1) using restriction fragment length polymorphism (RFLP) after polymerase chain reaction (PCR). Genotyping of CYP2E1 examined G-to-C transversions at nucleotide -1019 (Rsal), or C-to-T transition at nucleotide -1259 (PstI). Genotyping of NQO1 examined C-to-T transition (Hinfl). The presence of the GSTM1 and GSTT1 genes was detected using published methods (not described in detail).

STATISTICAL ANALYSIS

Group mean values were compared using Mann-Whitney U-test and ANOVA.

**Result:**

BENZENE EXPOSURE

Average benzene exposure was 9.3 ug/m<sup>3</sup> (7 hr TWA; geometric mean 6.7 ug/m<sup>3</sup>) or 3.7 ug/m<sup>3</sup> (7 hr TWA; geometric mean 3.4 ug/m<sup>3</sup>) for the outdoor- and indoor workers, respectively. There were no significant differences in blood benzene or urinary TMA or SPMA between the groups (although SPMA was increased in smokers compared to non-smokers)

DISTRIBUTION OF POLYMORPHISMS

Genotypic analysis of the population showed:

- 94% were homozygous for CYP2E1 (6% possessed a single mutant allele);
- 48% were homozygous for wild-type GSTM1 while 52% were homozygous for variant alleles for GSTM1;
- 76% were homozygous for wild-type GSTT1 while 25% were homozygous for variant alleles for GSTT1;
- 66% were homozygous for wild-type NQO1, 3% were homozygous for variant alleles for NQO1, and 31% were heterozygous (ie expressed both wild-type and mutant alleles)

EFFECT OF GENOTYPE ON EXCRETION OF TMA AND SPMA

TMA/blood benzene ratio was partially modulated by GST genotypes, with significantly higher values in null individuals (GSTM1 and GSTT1 combined). However a greater

fraction of the total variance of TMA/blood benzene in the study population was explained by independent variables such as season of sampling, smoking habits and gender.

Alterations in SPMA/blood benzene ratios were only associated with smoking and occupation, with no association with the metabolic polymorphisms assessed in this study.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

Metabolism of benzene to urinary trans, trans muconic acid and S-phenylmercapturic acid is affected only to a limited extent by genetic polymorphisms in individuals with low-level exposure to benzene. Gender, lifestyle and smoking have a larger impact on variability in these biomarkers.

04-MAR-2003

(1208)

**Endpoint:**

other: polymorphism of NAD(P)H:quinone oxidoreductase in humans

**Remark:**

This hypothesis-generating review article considers the possible role of genetic polymorphisms in expression of NAD(P)H:quinone oxidoreductase (NQO1) and other metabolic enzymes in benzene-induced bone marrow toxicity. NQO1 catalyses the reductive metabolism of endogenous and environmental quinones, including benzene quinone. The review is based upon a search of the literature (MEDLINE, PubMed) for references published between 1975-2001.

There are 22 reported (published) single-nucleotide polymorphisms in the NQO1 gene. The wild type (coded by the NQO1\*1 allele) predominates while the mutant NQO1\*2 allele shows a variable rate of expressed in different ethnic populations (caucasians = 22%; Asians = 45%). NQO1\*2 codes for a mutation that expresses negligible NQO1 activity. A large epidemiologic investigation has reported that individual homozygous for NQO1\*2 exhibit an up to 7-fold greater risk of bone marrow toxicity.

**Source:**

A.K. Mallett Surrey

06-SEP-2002

(818)

**Endpoint:** Hematotoxicity

**Method:** HUMAN HAEMATOPOIETIC PROGENITOR CELL ASSAYS  
Human cord blood mononuclear cells were isolated from cryopreserved samples (as described by Van Den Heuvel et al (1999) Toxicol In Vitro 13:605). Cells were plated out and incubated with benzene, phenol, catechol or hydroquinone for 14 days (37 degrees C, humidified 5% CO2 atmosphere). Proliferation of human granulocyte-macrophage precursor cells (hCFU-GM) was stimulated by addition of rhGM-CSF, while erythrocyte precursor cells (hBFU-E) were stimulated with erythropoietin.

MURINE HAEMATOPOIETIC PROGENITOR CELL ASSAYS  
Femoral bone marrow cells were obtained from 10-12 wk old female B6C3F1- or male Balb/c mice. Murine granulocyte-macrophage precursor cells (mCFU-GM) were stimulated with colony stimulating factor (CSF) derived from serum of mice injected with Salmonella endotoxin. After 7days exposure to benzene, phenol, catechol or hydroquinone the number of granulocyte and/or macrophage colonies was counted. In assays to compare the response of murine- and human cell lines, mCFU-GM cells were stimulated with rmGM-CSF and incubated under identical conditions to those of the human cell lines (see above).

MURINE STROMAL PROGENITOR CELL ASSAYS  
Adherent fibroblastoid cell colonies were counted after 10 d exposure to benzene, phenol, catechol or hydroquinone (using method of Schoeters et al., 1995, Toxicol In Vitro, 9:421; no further details given).

MURINE BONE-FORMING CULTURES  
Bone marrow cells were obtained from the flushed femora of 10-12 wk old female B6C3F1 mice. Cultures were evaluated for osteogenic differentiation after 4 wk growth in the presence of benzene, phenol, catechol or hydroquinone.

STATISTICS  
The concentration of benzene, phenol, catechol or hydroquinone that caused 50% inhibition in colony growth (IC50) was calculated using logistic regression. Statistical comparisons were made using Dunnett's test of one-way ANOVA.

**Result:** MURINE ASSAYS  
Benzene and phenol had hardly any effect on murine bone marrow cells (IC50 = or > 1600 uM), whereas catechol (IC50 in range 12-80 uM) and hydroquinone (IC50 in range 12-27 uM) gave a dose-related decrease in colony numbers and calcium deposition.

HUMAN CLONOGENIC ASSAYS  
The growth of hCFU-GM colonies from umbilical cord blood was impaired in a dose-dependent manner by hydroquinone (IC50 = 20 uM) and catechol (IC50 = 5 uM). (No results presented for benzene, phenol or catechol.)

## COMPARISON OF MURINE AND HUMAN CELL RESPONSES

Human CFU-GM (IC50 = 4 uM) were 6-fold more sensitive than murine CFU-GM (IC50 = 24 uM) to catechol. (No results presented for benzene, phenol or hydroquinone.)

**Source:**  
04-MAR-2003

A.K. Mallett Surrey

(1202)

**Endpoint:** Immunotoxicity

**Method:**

## SUBJECTS

Blood samples (peripheral venous blood) were collected from 25 healthy control subjects (5 men, 20 women; average age 38 yr; 20% smokers) and 9 donors with occupational exposure to benzene (8 men, 1 women; average age 43 yr; 22% smokers; no further information on benzene exposure provided). (Samples were also collected from small groups of individuals (n=10-13) with putative exposure to styrene, polycyclic aromatic hydrocarbons and 'mixed solvents'.)

## ANALYSIS OF SURFACE ANTIGENS

Heparinized whole blood was incubated with FITC, PE, PerCP or PAC labelled monoclonal antibodies active against lymphocyte surface antigens (abbreviations not defined by authors). After removal of erythrocytes, 5 monoclonal antibody combinations were employed to differentiate leukocyte populations using a FACSCalibur flow cytometer. Data for at least 10,000 leukocytes per samples were collected, and analysed using CELLQUEST Software 3.1.

## STATISTICAL METHODS

Statistical differences between control and exposed subjects were analysed using Student's T-test.

**Result:**

## LEUKOCYTE AND LYMPHOCYTE PARAMETERS

The mean leukocyte count (CALC 6.3/7), together with the contribution of lymphocytes (CALC 1.8/2.0) and neutrophils (CALC 4.1/4.7), were decreased non-significantly in the benzene-exposed group. (Comment: similar reductions were also present in cell counts from the other exposure groups, however statistical significance was achieved only sporadically (i.e. CALC 5.8/7 decrease in leukocytes, and CALC 3.7/4.7 reduction in neutrophils, in the "Mixed Solvents" group).)

Analysis of lymphocyte sub-populations showed no treatment-related effect on the relative contribution of T and B cells (all values from benzene-exposed donors within reference control range).

Benzene exposed individuals showed some significant changes lymphocyte phenotype, with an approx. doubling in HLA-DR+/CD3+ and CD71+/CD19+ lymphocytes, while CD62L+/CD3+ lymphocytes were decreased CALC 54.0/67.6. (Comment: expression of these phenotypes were also numerically increased (HLA-DR+/CD3+; CD71+/CD19+) or decreased (CD62L+/CD3+) in all of the other treatment groups, although the change was not always statistically significant.)

## CONFOUNDERS

Expression of HLA-DR+ T cells and CD71+ B cells was increased, and CD54+ B cells decreased, in subjects aged < 38 yr compared to cell populations for subjects aged > 38 yr. Smoking also correlated with other phenotypic- and SCE effects (although none that were altered in the benzene-exposed group).

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors conclude that benzene (and other aromatic hydrocarbons) activates peripheral lymphocytes and alters the incidence of CD25+/CD4+ T lymphocytes however the presence of confounding factors (age, smoking) makes the causal basis of this association uncertain.

25-JAN-2003

(107)

**Endpoint:** other: apoptosis in human bone marrow

**Remark:** Chinese language article, translation unavailable:

## ABSTRACT:

"The pathogenesis of hematol. chronic benzene intoxication patients was studied. Terminal deoxynucleotidyl transferase(TdT)-mediated dUTP nick end labelling (TUNEL) technique and immunohistochem. assay were employed resp. to detect apoptosis and proliferating cell nuclear antigen (PCNA) of human bone marrow cells in chronic benzene intoxication patients and healthy adult controls. Cell apoptosis index (AI) and PCNA index (PI) were calcd., and the correlation between AI and PI, as well as the correlation between AI, PI and other hematol. indexes (such as leukocyte count, neutrophil count, red cell count, Hb, platelet count) were also evaluated. The value of AI was 24.7%  $\pm$  12.0% and the value of PI was 10.7%  $\pm$  11.7%, resp. The differences of the AI and PI value between the benzene intoxication and control groups were statistically significant. However, no linear correlation between AI and PI was found in chronic benzene intoxication. Neither AI nor PI showed linear relationship with all the other hematol. indexes."

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors report evidence of accelerated apoptosis and inhibition of proliferation of bone marrow cells in subjects with chronic benzene intoxication. They suggest this may be linked to the pathogenesis of benzene-induced hematopoietic damage.

04-MAR-2003

(704)

**Endpoint:** Hematotoxicity

**Method:** SUBJECTS  
The study population comprised 97 'exposed' children (48 boys, 49 girls) living near a petrochemical 'estate' in Ulsan, Korea, and 95 'unexposed' children (46 boys, 49 girls) from a nearby suburban region. Approx. half of each sex/exposure group was age 8 yr, the remainder was age 11 yr. Each child completed a questionnaire on demographic characteristics, including information on medical history and exposure to passive smoking. (Comment: tabulated data presents a different (inconsistent) total number of boys and girls for the exposed and unexposed populations.)

#### EXPOSURE

Exposure estimates were based upon results from several ambient exposure studies:

1. Data for 1996 (referenced as Ulsan Metropolitan City, 1997; Korean language report). Benzene levels in the region of the petrochemical estate were 37.88 - 53.00 ppb, whereas those in the suburban region were 2.27 - 6.00 ppb. Levels of ethylbenzene, toluene and xylene were 15 - 2 fold higher in the petrochemical region than in the suburban region (no further details).

2. Data for 1997 (referenced as Na et al., 1998; Korean language publication) Benzene levels in the vicinity of the petrochemical plant 1.06 - 3.09 ppb compared to 0.08 - 1.66 ppb for the suburban area. Exposure to total VOCs (including aliphatic and aromatic hydrocarbons) was 1321 ppb around the petrochemical plant and 411 ppb in the suburban area. Levels of ethylbenzene, toluene, xylene and styrene were 1.1 - 3.7 fold greater than exposures in the suburban region.

3. Data for 1999 (referenced as Ministry of the Environment, 2001; Korean language report) Benzene levels were reported as 39.46 ppm in the region of the petrochemical estate and 2.43 ppb in the suburban region. Levels of ethylbenzene, toluene and xylene were 1.2 - 2.5 fold higher than in the suburban region.

#### HAEMATOLOGICAL ASSESSMENTS

Venous blood was collected between 09.00-11.00 hr (EDTA-vacutainer tubes) for determination of complete blood counts (CBC; comprising RBC, HgB and platelet counts) and WBC differential counts (both performed using Sysmex NE 8000 cell counter). A total of 192 blood samples were analysed in April, July and October 2000.

#### STATISTICAL METHODS

Students t-test was used to compare CBC values. Chi-squared was used to compare "general characteristics and proportion of two study groups". A repeated ANOVA was used to test for changes in CBC over time, using age and sex as covariates.

**Remark:** Although ambient exposure to benzene and other VOCs is the focus of this investigation, no contemporaneous exposure data were collected over the period of the study. It is also apparent that while differences in haematological parameters were present in children from the two regions, there was little consistency in the magnitude or direction of any of the reported changes. Temporal instability in data collected from the control population appeared to be quite marked.

**Result:** COMMENT: there appeared to be little consistency in haematological results obtained from exposed and unexposed children on the three timepoints included in this study.

In samples collected in April 2000, total WBC counts and absolute lymphocyte counts were significantly lower than control in 11 yr old children living near the petrochemical facility, while exposed 8 yr olds showed a significant decrease in RBC and HgB. Platelet counts were significantly lower in both exposed age groups at this time.

In July, total WBC, absolute neutrophil- and lymphocyte counts for 8 yr olds were significantly higher in the exposed group in comparison to the suburban group. There were no significant differences between exposed- and unexposed 11 yr olds at this sampling time.

In October, RBC counts and HgB values were significantly lower in 8 yr olds from the vicinity of the petrochemical plant, whereas platelet counts from the exposed group were higher than those of the controls. There were no significant differences between exposed- and unexposed 11 yr olds at this sampling time.

Analysis of CBC data using a generalised linear model analysis of variance showed that 'region', 'survey month', 'model' were significant independent variables influencing RBC and platelet counts, while sex and age influencing one or other of these measures. There was a significant association between 'region' or 'age' and WBC count, while age was also an independent variable influencing lymphocyte counts.

There was a strong, statistically significant interaction between [survey month x region and haematological parameters assessed in the study.

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors conclude that environmental exposure to VOCs containing low levels of benzene is associated with a higher prevalence of haematological abnormalities that could serve as biological markers for medical surveillance. The causal nature of this association is difficult to substantiate, however, since no concurrent measures of benzene exposure were included in the investigation.

27-MAR-2003

(677)

**Endpoint:** other: human genetic polymorphism and benzene metabolism

**Method:** SUBJECTS AND METHODS  
Benzene exposure (personal diffusive sampling with subsequent analysis by GC-FID) was assessed over the course of a work week (Monday morning to Friday evening; approx. 110 hr) for 40 subjects (20 men, 20 women, age 27-46 yr) living and working in central Copenhagen. Ten of the subjects worked partly outdoors (e.g. policemen), the remainder in an office environment. They answered a questionnaire that included questions of possible sources of benzene exposure, smoking habits, type of work etc.

A sample of venous blood (heparinised) and a 24 hr urine sample were collected from all subjects on day 1 of the study.

#### ANALYSIS OF 8-OXOdG AND COMET ASSAY

Washed lymphocytes were isolated by centrifugation, lysed and DNA isolated using published methods (Wellejus and Loft (2002) FASEB J 16:195). The concentration of 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) in lymphocyte DNA and in urine was then determined using HPLC with electrochemical detection.

A separate sample of lymphocytes was processed for single-cell electrophoresis (comet assay; Moller et al. (2001) FASEB J 15: 1181) after enzymic digestion (fapyguanine glycosylase, endonuclease). The cells were scored visually for damage (0 = no damage; 4 = maximum damage) and summed (giving a total score between 0 and 400).

#### BIOMARKERS OF BENZENE EXPOSURE IN URINE

Trans,trans muconic acid (t,t-MA) and S-phenylmercapturic acid (S-PMA) were quantified using GC-MS.

#### DETERMINATION OF GSTT1, GSTM1, GSTP1 AND NQO1 GENOTYPES

DNA was isolated from lymphocytes (phenol extraction) and used as a template for polymerase chain reaction (2 cycles) prior to agarose gel electrophoresis. After staining with ethidium bromide and visualisation with UV light, the genotypes GSTT1 (Pemble et al. (1994) Biochem J 300, 271), GSTM1 (Zhong et al. (1991) Carcinogenesis 12, 1533), GSTP1 (Harries et al. (1997) Carcinogenesis 18, 641) and NQO (Wiencke et al. (1997) Cancer Epidem Biomarker Prev. 6, 87) were determined by PCR based assays, following published methods.

#### STATISTICAL METHODS

Demographic data, urinary 8-oxodG and exposure data were analysed according to gender using Mann-Whitney tests. Differences in t,t-MA and S-PMA excretion according to NQO, GSTP1, GSTM1 and GSTT1 genotype status were analysed using multifactorial ANOVA. Analyses for 8-oxodG in lymphocytes and results from the comet assay were evaluated using

**Result:** Spearmans rank correlation test.  
Overall median benzene exposure was 2.53 ug/m<sup>3</sup>. The 10 subjects that worked partly outdoors were exposed to 3.6 ug/m<sup>3</sup> benzene but this was not significantly different from that for the 30 subjects with office duties (2.5 ug/m<sup>3</sup>; P = 0.06). There were 4 smokers among the subjects, with a median benzene exposure of 3.9 ug/m<sup>3</sup> (non-significant when compared with overall or sub-group median exposures).

There were no significant differences with respect to excretion of biomarkers between the two study populations, although S-PMA excretion in the outdoor workers (median 8.7 ug/24 hr) was (non-significantly) greater than that of office workers (3.7 ug/24 hr).

Excretion of t,t-MA was significantly greater for men (133 ug/24 hr) compared to women (65 ug/24 hr; P = 0.003), and was significantly associated with benzene exposure in men only (P = 0.025).

There was a significant correlation (P = 0.04) in 28 of the subjects between excretion of S-PMA and 8-oxodG in lymphocytes. Within this sub-population, there was a slightly greater correlation between S-PMA and 8-oxodG in subjects carrying the NQO1+/- genotype (R = 0.58, P = 0.08, n = 10) than in subjects with the NQO+/+ genotype (R = 0.33, P = 0.17, n = 18).

Multifactorial ANOVA indicated expression of the heterozygous NQO+/- genotype was a significant predictor of both S-PMA (P = 0.011) and t,t-MA (P = 0.004) in urine compared to subjects with wildtype NQO+/+. The GSTP1, GSTM1 and GSTT1 genotypes did not affect excretion of t,t-MA or S-PMA significantly (although subjects with wildtype GSTM1 excreted more t,t-MA than subjects that were GSTM1 null).

Excretion of t,t-MA was increased in subjects expressing wildtype forms of both GSTM1 and GSTT1 (additive effect, median excretion 168 ug/24 hr, n = 18) when compared with subjects that were null for GSTM1 (with wildtype GSTT1), or null for GSTT1 (with wildtype GSTM1) or null for both (median 84 ug/24 hr, n = 42, P = 0.004). No comparable effect present for S-PMA in urine.

**Source:** A.K. Mallett Surrey

**Conclusion:** Excretion of the benzene metabolites t,t-muconic acid and S-phenylmercapturic acid is modified by genotypic variations in the expression of NADP(H):quinone oxidoreductase and glutathione-S-transferases. This may influence oxidative DNA damage in lymphocytes following exposure to benzene.

27-APR-2004

(1107)

**Endpoint:** other: AhR knock-out mice and benzene-induced haematotoxicity

**Method:** ANIMALS AND TREATMENTS  
Aryl hydrocarbon receptor knock-out mice (AhR<sup>-/-</sup>) were mated with C57BL/6 mice over 6 generations to produce AhR<sup>+/+</sup>, AhR<sup>+/-</sup> and AhR<sup>-/-</sup> genotypes (confirmed by polymerase chain reaction screening of DNA). In one series of studies, animals (age 8 wk, n=5-7) were exposed to 300 ppm benzene vapour (6 hr/d, 5 d/wk) for 2 wk. In another series, mice were treated with phenol (PH; 75 mg/kg bwt) and hydroquinone (HQ; 75 mg/kg bwt) by i.p. injection (saline) twice daily at 6 hr intervals for 3 days (calculated as equivalent to 309 ppm benzene vapour, assuming 100% uptake and metabolic conversion).

#### BLOOD AND BONE MARROW PARAMETERS

Haematological parameters (WBC, RBC, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin) were quantified on a sample of peripheral blood collected from the orbital sinus while bone marrow cellularity was assessed using cell suspensions prepared from femoral marrow. All determinations employed automated counting systems.

Granulocyte-macrophage colony forming units (CFU-GM; lymphocyte progenitor cells) were cultured in semisolid methyl cellulose culture for 6 days prior microscopic manual counting.

#### WESTERN BLOT ANALYSIS FOR P21 AND CYP2E1

Protein extracts from femoral bone marrow cells (Yoon et al. (2001) Exp Hematol 29, 278) and liver (Valentine et al. (1996) Toxicol Appl Pharmacol 141, 205) were prepared using published methods. After denaturation and SDS polyacrylamide gel electrophoresis, the samples were transferred to a polyvinylidene fluoride membrane, incubated with rabbit polyclonal antibody for p21 analysis or goat anti-rat polyclonal antibody for CYP2E1, then conjugated with horseradish peroxidase secondary antibody. Band densities were measured using an image analyser.

#### REVERSE TRANSCRIPTASE PCR FOR AhR mRNA

Total RNA was extracted from liver or bone marrow cells from AhR<sup>+/+</sup> mice (using a preparatory kit: ISOGEN), and reverse transcribed prior to PCR amplification with oligonucleotide primers specific for the mouse Ah receptor (28 cycles for liver, 30 cycles for bone marrow). The amplified samples were subject to agarose gel electrophoresis.

#### STATISTICAL METHODS

Results were analysed using two-tailed Student t-tests.

**Result:** BLOOD AND BONE MARROW PARAMETERS FOLLOWING BENZENE EXPOSURE  
Peripheral blood parameters and bone marrow cellularity were decreased significantly in AhR<sup>+/+</sup> and AhR<sup>+/-</sup> mice following 2 wk exposure to 300 ppm benzene compared with values from

AhR<sup>-/-</sup> mice (values below obtained by interpolation from graphical results; presented as percentage of unexposed controls):

	AhR <sup>+/+</sup>	AhR <sup>+/-</sup>	AhR <sup>-/-</sup>
WBC	~40% (P<0.05)	~45% (P<0.05)	~80% (ns)
RBC	~80% (P<0.05)	~80% (P<0.05)	~100%
HGB	~85% (P<0.01)	~90% (P<0.05)	~105%
HCT	~85% (P<0.01)	~90% (P<0.05)	~105%

Bone marrow cellularity was decreased significantly in all 3 phenotypes (P<0.05) following exposure to 300 ppm benzene for 2 wk, however the magnitude of response (presented as percentage of control) was less in AhR<sup>-/-</sup> mice:

AhR <sup>+/+</sup>	49.4%
AhR <sup>+/-</sup>	53.8%
AhR <sup>-/-</sup>	81.6%

The presence of CFU-GM per femur was similarly decreased (P<0.05) in AhR<sup>+/+</sup> or <sup>+/-</sup> mice, but was unaffected in the AhR<sup>-/-</sup> phenotype:

AhR <sup>+/+</sup>	28.3%
AhR <sup>+/-</sup>	52.0%
AhR <sup>-/-</sup>	~90.0%

#### BLOOD AND BONE MARROW PARAMETERS FOLLOWING TREATMENT WITH PHENOL AND HYDROQUINONE

Treatment with PH and HQ resulted in toxicity with the death of one AhR<sup>+/-</sup> mouse while one AhR<sup>-/-</sup> mouse was moribund 3 d post-injection. In survivors, the number of WBC and cellularity of bone marrow was decreased significantly (P<0.05) in all three groups:

	AhR <sup>+/+</sup>	AhR <sup>+/-</sup>	AhR <sup>-/-</sup>
WBC	58%	30%	26%
Bone marrow	29%	26%	26%

Haemoglobin and haematocrit values were decreased (P<0.05) in AhR<sup>-/-</sup> mice (91% and 89%, respectively, of control) but unaffected in AhR<sup>+/+</sup> or AhR<sup>+/-</sup> phenotypes.

#### EXPRESSION OF P21, CYP2E1 AND AhR mRNA AFTER BENZENE EXPOSURE

Western blot analysis showed that p21 was upregulated in AhR<sup>+/+</sup> and AhR<sup>+/-</sup> mice (but not AhR<sup>-/-</sup>) mice following 2 wk exposure to benzene.

Expression of CYP2E1 in livers from AhR<sup>+/+</sup> mice after exposure to 300 ppm benzene was approx. double that of controls (P<0.05), while AhR<sup>-/-</sup> mice exhibited only a smaller (approx. 50%; non-significant) increase.

Expression of AhR mRNA was increased by approx. 50% in the liver of benzene-exposed AhR<sup>+/+</sup> mice (non-significant).

#### Source:

A.K. Mallett Surrey

#### Conclusion:

Benzene (300 ppm, 2 wk) was not haematotoxic in aryl hydrocarbon receptor knockout mice (AhR<sup>-/-</sup>), with no changes in cellularity of peripheral blood or bone marrow,

or in levels of granulocyte-macrophage colony forming units in bone marrow, or expression of p21. The results indicate that AhR mediates benzene haematotoxicity, at least in part, through the induction of CYP2E1.

08-APR-2004

(1301)

**Endpoint:** other: formation of adducts in mice and rats (i.p. exposure)

**Method:** ANIMALS AND TREATMENTS  
Male B6C3F1, DBA/2 and C57BL/6 mice (30 g) and male F344/Sim rats (200 g) were used in these studies. Animals received a single i.p. injection of <sup>14</sup>C benzene in corn oil (0.1 uCi in 200 ul corn oil) and were killed (carbon oxide) 0-48 hr later. Comment: These strains/species were selected since they had been used previously in cancer/genotoxicity studies and appear to differ in susceptibility to benzene toxicity.

#### SAMPLE COLLECTION AND HPLC ANALYSIS

Liver was sampled and immediately placed on dry ice and stored frozen. Bone marrow was collected from femur and humerus and stored (as above).

#### ISOLATION OF DNA AND PROTEIN FROM TISSUE SAMPLES

DNA was isolated from liver or bone marrow after solubilisation and RNase treatment, followed by precipitation with isopropyl alcohol. This procedure gave approx. 100 ug DNA per 100 mg of starting tissue. Protein was precipitated with perchloric acid after solubilisation, and protein content determined using the Bradford microassay.

#### ACMS ANALYSIS

The radiocarbon content of the protein and DNA extracts was determined using accelerator mass spectrometry (AMS) after converting dried samples to graphite and subsequent analysis using published procedures (Turtletaub et al. (1993) Postlabeling Methods for Detection of DNA Adducts, IARC, Lyon, pp 293-300). Comment: AMS measures the ratio <sup>14</sup>C relative to <sup>13</sup>C, which was then normalised to <sup>14</sup>C/<sup>12</sup>C using a standard carbon source. The ratios were converted to mass of <sup>14</sup>C benzene based upon the specific activity of the benzene following correction for background.

#### STATISTICAL METHODS

Z tests were used to examine for differences between AUCs for adduct formation.

**Result:** MACROMOLECULAR ADDUCTS IN RATS AND MICE

#### LIVER

DNA and protein adducts in liver peaked within 0.5 to 1 hr, while adduct levels in rat liver continued to increase until 6-12 hr post-dosing. Representative results (pg <sup>14</sup>C-benzene adduct per g protein or DNA) are given below:

Protein adducts

Time (hr)	<0.08	0.5	1	6	12	24	48
B6C3F1	2	2440	1640	1000	1050	1040	290
DBA/2	8	2000	1650	1200	430	410	320
C57BL/6	0	1660	670	190	200	200	170
F344	2	150	320	590	610	480	279

## DNA adducts

Time (hr)	<0.08	0.5	1	6	12	24	48
B6C3F1	3	170	100	60	70	60	60
DBA/2	110	260	200	150	140	130	140
C57BL/6	3	40	30	30	30	30	30
F344	1	10	20	40	50	40	20

## BONE MARROW

Bone marrow kinetics were similar in rats and mice. Representative results (pg 14C-benzene adduct per g protein or DNA) are given below:

## Protein adducts

Time (hr)	1	6	12	24	48
B6C3F1	830	830	1120	820	690
DBA/2	370	500	640	340	290
C57BL/6	270	330	340	280	160
F344	30	240	290	170	160

## DNA adducts

Time (hr)	1	6	12	24	48
B6C3F1	70	140	230	110	90
DBA/2	30	40	150	120	60
C57BL/6	20	70	80	110	70
F344	10	30	30	20	10

In all cases, protein adduct levels in liver and bone marrow were 10-20 fold greater than levels of DNA adducts.

## AUCs FOR ADDUCTS IN LIVER AND BONE MARROW

Adduct levels present 0-48 hr post-dosing were used to calculate AUCs and provide a measure of total reactive dose present in liver and bone marrow from the different mouse strains and rodent species.

AUCs for bone marrow DNA and protein adducts in the B6C3F1 mouse were approx. 7 fold and 4 fold greater, respectively, than levels in the rat. AUCs for bone marrow protein adducts were approx. 3-10 fold greater than mean DNA adduct AUCs (depending on strain and species).

AUCs for liver adducts differed among the mouse strains and rodent species (no consistent pattern present). With background correction, AUCs for liver protein and DNA adducts were ordered identically among the mouse strains (B6C3F1 > DBA/2 > C57BL/2), however the relative order of the rat was variable (positioned between DBA/2 and C57BL/6 for protein adducts; between B6C3F1 and DBA/2 for DNA adducts).

AUC (corrected for background):

	B6C3F1	DBA/2	C57BL/6	F344
Bone marrow protein	21900	17800	12100	9100
Bone marrow DNA	4600	4800	4100	900
Liver protein	36700	26300	10600	21700
Liver DNA	2800	1500	1400	1800

Comment: A different relative order was obtained for DNA adducts in bone marrow when uncorrected AUCs were compared among strains/species:

Bone marrow DNA, corrected: DBA/2 > B6C3F1 > C57BL/6 > F344

Bone marrow DNA, uncorrected: B6C3F1 > DBA2 > C57BL/6 > F344

Similarly, a different relative order was also obtained for uncorrected AUCs for DNA adducts in liver:

Liver DNA, corrected: DBA/2 > B6C3F1 > F344 > C57BL/6

Liver DNA, uncorrected: DBA/2 > B6C3F1 > F344 > C57BL/6

**Source:**

A.K. Mallett Surrey

**Conclusion:**

AUCs for protein and DNA adducts in bone marrow, the primary target for benzene toxicity, decreased in the following order: B6C3F1 or DBA/2 mouse, then C57BL/6 mouse, F344 rat. Similarly, adduct AUCs in liver were greater in B3C3F1 mice than in F344 rats (although the trend for DBA/2 and C57BL/6 mice was less clear). The findings may be linked with the differing ability of these various mouse strains and rodent species to metabolise benzene.

08-APR-2004

(1171)

**Endpoint:**

other: formation of adducts in mice and rats  
(inhalation exposure)

**Method:**

## ANIMALS AND TREATMENTS

Male B6C3F1 mice (30 g) and male F344/Sim rats (200 g) were exposed to 14C benzene via inhalation. Rats were exposed nose-only to 0.1 ppm 14C-benzene for 20 min (0.09 uCi/animal), then housed in polycarbonate chambers under negative airflow. Mice were exposed nose only to 0.1 ppm for 2.5 min, then returned to their cages and kept under negative pressure. The exposure regime for both species was intended to deliver a systemic dose of 5 ug/kg bwt. Groups of rats and mice were sacrificed (carbon oxide) at 0, 0.16, 0.5, 1, 3, 12 and 24 hr post-exposure.

## SAMPLE COLLECTION AND HPLC ANALYSIS

Livers were sampled and immediately placed on dry ice and stored frozen. Bone marrow was collected from femur and humerus and stored.

## ISOLATION OF DNA AND PROTEIN FROM TISSUE SAMPLES

DNA was isolated from liver or bone marrow after solubilisation and RNase treatment, followed precipitation with isopropyl alcohol. This procedure gave approx. 100 ug DNA per 100 mg of starting tissue. Protein was precipitated with perchloric acid after solubilisation, and protein content determined using the Bradford microassay.

## ACMS ANALYSIS

The radiocarbon content of the protein and DNA extracts was determined using accelerator mass spectrometry (AMS) after converting dried samples to graphite and subsequent analysis using published procedures (Turtletaub et al. (1993) Postlabeling Methods for Detection of DNA Adducts, IARC, Lyon, pp 293-300). Comment: AMS measures the ratio  $^{14}\text{C}$  relative to  $^{13}\text{C}$ , which was then normalised to  $^{14}\text{C}/^{12}\text{C}$  using a standard carbon source. The ratios were converted to mass of  $^{14}\text{C}$  benzene based upon the specific activity of the benzene after correction for background.

#### STATISTICAL METHODS

Z tests were used to examine for differences between AUCs for adduct formation.

#### Result:

#### MACROMOLECULAR ADDUCTS IN RATS AND MICE BY AUC

DNA and protein adducts were present in liver and bone marrow of both rodent species, with levels of protein adducts greater than levels of DNA adducts regardless of the tissue examined.

In mice, protein and DNA adduct levels peaked in liver 0.5-1 hr after exposure, and in bone marrow 12-24 hr post-exposure. In rats, protein and DNA adduct levels in liver peaked within 1 hr whereas no clear pattern was apparent in bone marrow. Representative results (pg  $^{14}\text{C}$ -benzene adduct per g protein or DNA) are given below:

#### Adducts in liver

	Mouse		Rat	
	Protein	DNA	Protein	DNA
0.08 hr	370	10	70	70
0.5 hr	2000	90	120	60
1 hr	1700	80	30	20
12 hr	800	30	40	20
24 hr	860	20	30	10

#### Adducts in bone marrow

	Mouse		Rat	
	Protein	DNA	Protein	DNA
0.08 hr	5	5	50	-10
0.5 hr	430	--	40	5
1 hr	--	--	50	10
12 hr	670	200	40	-10
24 hr	480	440	30	10

#### AUCs FOR ADDUCTS IN LIVER AND BONE MARROW

Adduct levels present 0-24 hr post-exposure were used to calculate AUCs and provide a measure of total reactive dose present in liver and bone marrow. Levels in the mouse were consistently greater than levels in the rat.

#### AUC:

	B6C3F1	F344
Bone marrow protein	10300	930
Bone marrow DNA	6300	270
Liver protein	25300	1000

**Source:** Liver DNA 1000 480  
A.K. Mallett Surrey

**Conclusion:** Adduct levels in DNA and protein were consistently greater in the mouse than in the rat exposed to 0.1 ppm 14C-benzene for 2.5 or 25 minutes, respectively (exposures designed to deliver the equivalent to 5 ug/kg bwt to both species).

14-APR-2004 (1171)

**Endpoint:** other: human genetic polymorphism and benzene poisoning

**Method:** SUBJECTS AND METHODS

One hundred and fifty six patients diagnosed as having benzene poisoning were recruited from four regions of China. Benzene poisoning was characterised by a consistent total white cell count between 4000-4500 per ul and a consistent platelet count < 80,000 per ul over several months with at least 6 mo documented exposure to benzene after exclusion of other potential causes of abnormal blood counts (e.g. exposure to radiation or drugs). The control population consisted of 152 workers with occupational exposure to benzene but who did not exhibit symptoms of benzene poisoning; the controls were matched to the patients in terms of age, sex and duration of working with benzene. General information about the subjects was obtained by interview. Subjects who smoked at least one cigarette per day for more than 1 yr were classified as smokers.

#### EXPOSURE ESTIMATES

Individual exposure to benzene was estimated based upon monitoring data (controls) or an evaluation of probable exposure (patients; based upon long-term work place records) at the time that a diagnosis of benzene poisoning was made.

#### BLOOD ANALYSES

Genomic DNA was extracted from frozen (-80 degrees C) blood using phenol-chloroform. Samples were amplified for 35 cycles using a polymerase chain reaction method, subjected to automated sequencing and separated using denaturing HPLC. Restriction fragment length polymorphism and PCR were used to detect polymorphism on the promoter and complete coding regions of NQO3 (NADPH-quinone oxidoreductase), CYP2E1 and MPO (myeloperoxidase) and the null genotypes of GSTM1 (glutathione-S-transferase mu-1) and GSTT1 (glutathione-S-transferase theta-1) using published methods.

#### STATISTICAL METHODS

Chi-square and Fishers exact test was used to examine the association between genetic polymorphisms and individual susceptibility to benzene haematotoxicity. Homogeneity of odds ratios was examined using the Breslow-Day method. Logistic regression was used to examine the relationship between intensity and duration of exposure and benzene poisoning.

**Remark:** The authors suggest that genetic polymorphisms in genes encoding for enzymes involved in the metabolism of benzene

might be responsible for differences in human susceptibility to poisoning by benzene, and cite the following in support of this hypothesis:

- \* NQO1 with a C to T substitution in cDNA at nucleotide 609 is associated with decreased or absent enzyme activity;
- \* Individuals with the NQO1 T/T genotype and high levels of CYP2E1 exhibit an increased sensitivity to benzene poisoning;
- \* A single nucleotide polymorphism (substitution of A for G) in the c.-463 promoter gene may decrease expression of MPO;
- \* Individuals with a GSTT1 null genotype exhibit increased susceptibility to myelodysplastic syndrome.

**Result:**

There was no significant difference between the patients and the controls with regard to age, exposure duration and intensity of exposure.

**GENETIC POLYMORPHISMS**

A random sample of 24 patients and 24 controls were examined for possible genetic variations in NQO1, CYP2E1 and MPO genes using direct sequencing. Seventeen single nucleotide polymorphisms (SNPs) were identified in the screened regions of NQO1, CYP2E1 and MPO, and 6 SNPs in CYP2E1 and MPO genotyped in all subjects using denaturing HPLC. Comment: The publication contains extensive supporting information on sequence variation, allele frequency and genotype frequency.

**EFFECTS OF GENETIC POLYMORPHISMS ON BENZENE POISONING**

The distribution of 8 independent polymorphisms was compared in patients and controls, however there was no association between genetic polymorphism and susceptibility to benzene poisoning for the two groups. The frequency of patients with 2 copies of a variation in NQO1 (25.7%) was slightly higher than the controls (19.6%), however this difference was not statistically significant. There was little difference in odds ratio when adjusted for sex and exposure duration and intensity.

**MODIFICATION OF RISK OF BENZENE POISONING BY LIFESTYLE AND GENETIC POLYMORPHISM**

The frequency of genotype NQO1 c.609 T/T in regular smokers was 44.4% for patients and 9.7% for controls (P = 0.01). There was a 7.73-fold increased risk of benzene poisoning for smokers carrying this gene than for those with the heterozygous or wild type gene. Adjustment for sex, exposure duration/intensity and alcohol consumption had minimal impact on the results.

Smokers carrying the CYP2E1 c.-1293G/C or C/C genotypes had a 3.3-fold increase in risk of benzene poisoning compared with smokers carrying the wild type gene (G>C or G/G) however this was no-longer apparent after adjustment for sex, exposure duration/intensity and alcohol consumption.

Among alcohol drinkers, NQO1 c.609 T/T occurred in 61.1% of the patients compared with 12.5% of the controls. Alcohol drinkers with NQO1 c.609 T/T had an 11-fold greater risk of benzene poisoning than alcohol drinkers with a NQO1 c.609

C/T or C/C genotype (P = 0.005), which increased further (21-fold) after adjustment for sex, duration/intensity of exposure and cigarette smoking.

The frequency of patients with GSTM1 null genotype was higher among alcohol drinkers than in controls (66.7% versus 33.3%), however this was not statistically significant. Alcohol drinkers that were GSTM1 null were 4.2 fold more at risk of benzene poisoning than alcohol drinkers that were GSTM1 non-null.

#### MULTIPLE VARIABLE UNCONDITIONAL LOGISTIC REGRESSION

There was an apparent joint action between alcohol consumption and NQO1 c.609 C>T (P = 0.007), and between alcohol consumption and NQO1 c.609 C>T, CYP2E1 c.-1293G>C and GSTT1 null genotypes (P = 0.019).

Individuals with NQO1 c.609 T/T, CYP2E1 c.-1293 C/C or C/G and GSTT1 null were 5.6 fold more susceptible to benzene poisoning than individual carrying NQO1 c.609 C/T OR C/C, CYP2E1 c.-1293 G/G and GSTT1 non-null genotypes.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The results suggest that an interaction between polymorphisms in NQO1, CYP2E1 and GSTT1 genes and lifestyle factors (alcohol, smoking) may influence the risk of benzene poisoning.

20-APR-2004

(1228)

**Endpoint:**

other: neuromodulatory effect

**Study described in chapter:** 5.4 Repeated Dose Toxicity

**Species:**

rat

**Strain:**

Sprague-Dawley

**Sex:** male

**Route of administration:**

ip

**No. of animals:**

10

**Exposure Period:**

3 day(s)

**Frequency of treatment:**

daily

**Doses:**

0,5/kg bw/d

**Control Group:**

yes

**Test substance:**

as prescribed by 1.1 - 1.4

**Result:**

The activity of aminopeptidase, which is proposed to regulate the activity of several neuroactive peptides, was measured by the hydrolysis rate of Lys- and Leu-2-naphthylamides in several brain regions. Both enzyme activities were decreased in the thalamus, hypothalamus, hippocampus, and amygdala after benzene treatment suggesting that central neuropeptide transmission may be activated.

**Source:**

German Rapporteur

**Flag:**

Risk Assessment

06-JUL-2005

(279)

**Endpoint:** other: Neuromodulatory effect  
**Study described in chapter:** 5.4 Repeated Dose Toxicity  
**Species:** mouse  
**Strain:** other: Kunming **Sex:** male  
**Route of administration:** inhalation  
**Exposure Period:** 30 day(s)  
**Frequency of treatment:** 2 hours/daily  
**Doses:** 0.78, 3.13, 12.52 ppm  
**Control Group:** yes

**Method:** other

**Method:** 10 animals/group

**Result:** Neurobehavioural function and changes in acetylcholinesterase (AChE) activity at low level exposure to benzene were investigated. Adult male Kunming mice were exposed to 0, 0.78, 3.13, and 12.52 ppm of benzene for 2 hours per day for 30 days. Neurobehavioural function was measured in tests on the limb grip strength, rapid response (learning/memory functions) and locomotor activity. Depressed neurobehavioural function was recorded at the high dose level, whereas the mid dose animals did not show consistent effects and low dose animals responded with higher activities compared to the controls. Decreases of AChE activity in blood and brain were noted with increase of benzene exposure dose, especially in the mid and high dose groups. Values of AChE activity only gained significance in the brain of the high dose group.

**Source:** German Rapporteur

**Flag:** Risk Assessment

06-JUL-2005

(687)

### 5.10 Exposure Experience

**Remark:**

BONE MARROW DEPRESSION

Bone marrow depression results in a spectrum of haematological changes depending upon the degree of exposure and to individual susceptibility. Thrombocytopaenia, pancytopaenia, and aplastic anaemia may result.

Aksoy (1972) reported on 32 individuals who had been exposed to 150-650 ppm benzene for periods of 4 months-15 years and who had pancytopaenia with hypoplastic, hyperplastic or normoplastic marrow. Eight of the 32 were thrombocytopaenic with associated haemorrhage and infection. Severe or fatal aplastic anaemia seems to be associated with high exposure (>200 ppm) (Aksoy, 1972; Vigliani & Forni, 1976).

Much of the literature on haematological effects of benzene relates to exposures that took place in the 1930s and 1940s and consequently had poor characterization of benzene exposure (WHO, 1993). The haematotoxic effects of benzene appear to occur with exposures to >30 ppm (ECETOC, 1984).

More recent studies show no (or marginal effects) after 8 hour TWA exposures of 2-30 ppm, <0.1-25 ppm, <1.0-10 ppm and 0.01-1.4 ppm (Townsend et al. 1978; Tsai et al. 1983; Yardley-Jones et al. 1988; Collins et al. 1991).

As benzene is toxic to bone marrow it would not be unexpected that it could have an effect on immune function.

Studies of immune function in workers have however usually been compromised by confounding exposures (WHO, 1993).

**Source:**

BP Chemicals Ltd LONDON

German Rapporteur

**Flag:**

Risk Assessment

07-JUL-2005

(19) (224) (320) (1149) (1161) (1216) (1252) (1292)

**Remark:**

PETROLEUM INDUSTRY WORKERS

A number of cohort studies have investigated oil refining and petroleum distribution workers but few have focussed specifically on benzene exposure.

A large cohort study of British refinery workers (34,781 workers employed for at least a year between 1950-1975) showed no excess mortality from leukaemia (Rushton & Alderson, 1981a). No benzene exposure measurements were made but when leukaemia cases and controls were qualitatively assigned to low, medium or high benzene exposure groups on the basis of job category in a nested case-control study, a significant association ( $p=0.05$ ) was reported between leukaemia and combined high or medium exposure compared to the low exposure group ( $RR=3.0$ , 95%  $CI=1.2-7.2$ ) (Rushton & Alderson, 1981b).

The 39 year follow up of this study has shown that in 34,569 refinery workers there was still no leukaemia excess (68 observed compared to 70 expected). In the follow up of the 23,306 distribution workers there were 61 leukaemias observed compared to 57 expected ( $SMR=108$ , 95%  $CI=83-140$ ) (Rushton, 1993).

The effects of occupational exposure to lower concentrations of benzene has been the subject of much study. The availability of data from further large cohorts occupationally exposed to benzene is relevant to defining the dose effect relationship at well characterized low exposures. Such data is likely to be forthcoming from the UK oil distribution cohort. This is currently the subject of a nested cohort study, due to report early in 1995.

Tsai et al. (1983) reported on 454 US refinery workers employed from 1952 until 1978 and found no deaths from leukaemia (0.4 expected). Personal samples indicated however that during the period 1973-1982 only 16% of 1394

samples indicated exposure to air concentrations (TWA) of >1 ppm.

Another US refinery study was apparently set up because of a suspected excess of leukaemia in the work force (McCraw et al. 1985). Fourteen leukaemia deaths were observed (6.6 expected) of which 8 were acute myeloid leukaemia (2.0 expected). A later nested case-control study found no association with potential exposure to benzene (Austin et al. 1986).

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(61) (750) (976) (978) (979) (1161)

**Remark:** COKE OVEN WORKERS

A number of cohort studies have looked at mortality in coke oven workers. No excesses of leukaemias and lymphomas combined or of leukaemias respectively were found in North American or Dutch coke oven workers (Redmond et al. 1972; Swaen et al. 1991). Exposures to benzene were not reported in these studies.

Preliminary results from a total 6520 British male coke plant workers have been reported (Hurley et al. 1991). Personal air sampling from two cohorts of benzole workers (84 and 307 workers respectively) showed benzene concentrations of <0.19-15 ppm. Contemporary mean 8 hour time weighted averages for similar workers in the UK during the 1980s were 1.3 ppm. No increased risk of mortality from leukaemia has been observed in either cohort (SMR=98, 95% CI=2-429, 1 death and SMR=76, 95% CI=1-192, 1 death). For two cohorts of coke oven workers (total 2349 workers) leukaemia mortality had SMRs of 34, 95% CI=0-186, 1 death and 35 95% CI=1-192, 1 death respectively.

**Source:** BP Chemicals Ltd LONDON  
German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(541) (931) (1103)

**Remark:** ACUTE TOXICITY

The acute toxicity to humans when benzene is inhaled has been described as being as follows (Gerarde, 1960):

20,000 ppm 5-10 minutes exposure can give fatalities  
7,5000 ppm 30 minutes exposure may be fatal  
1500 ppm 60 minutes exposure causes serious symptoms  
500 ppm 60 minutes exposure leads to symptoms of illness  
50-150 ppm 5 hours exposure causes headache, lassitude and weakness  
25 ppm 8 hours exposure causes no clinical effect.

Signs of toxicity following exposures are CNS depression, cardiac arrhythmia and respiratory failure resulting in death. At lower levels of exposure CNS effects are rapidly reversible.

The acute lethal dose of benzene to humans when ingested has been estimated to be about 10 ml (8.8 g). The signs and symptoms of toxicity are typically those of narcotic solvent type materials leading to profound CNS depression, collapse and death (WHO, 1993).

Avis & Hutton (1993) reported of 3 fatalities of acute benzene poisoning. Victims were exposed to benzene fumes for a few minutes after opening a valve on a chemical cargo ship. Findings at autopsy: 2nd degree chemical burns to face, trunk and limbs (20-70% burn), hemorrhagic airless lungs with alveolar hemorrhage and pulmonary edema, brains with vascular congestion, benzene concentration in blood 30-120 mg/l and in brain 58-63 mg/l.

**Source:**

Deutsche Shell Chemie GmbH Eschborn, German rapporteur  
German Rapporteur

**Flag:**

07-JUL-2005

Risk Assessment

(62) (402) (1252)

**Remark:**

Partly on the basis that it is a study in which benzene exposures appear not to have been confounded by other significant chemical exposures, the Rinsky et al. 1987 study has been widely cited. It has also been the basis of a number of quantitative risk assessment extrapolations which have attempted to project the leukaemia risk of benzene. However, this study has a number of limitations, largely concerning the estimation of worker exposure to benzene. No industrial hygiene data were available for the period 1940-1946 during which 6/9 leukaemia case workers were employed. It has been suggested that the Rinsky et al. 1987 estimates of exposure prior to 1946 were severe underestimates because of the following factors (Paustenbach et al. 1992; Crump & Allen, 1984; Cody et al. 1993; CEFIC 1988):

An extensive exhaust ventilation system was installed at one plant in 1946. Exposures would have been expected to have been higher without such a system than were subsequently shown by industrial hygiene monitoring and used as a basis for determining worker exposures.

Although mortality from aplastic anaemia was not reported in

the study, blood dyscrasias including aplastic anaemia were not uncommon amongst the work force at the Pliofilm plants indicating that some high exposures had taken place.

Review of worker haematological data and comparison with individual "pre-employment" data suggest that workers in the 1940s experienced clinically detectable bone marrow depression during their initial period of work in benzene areas of the plant. This information supports the view that benzene exposures were higher during this period than those estimated by Rinsky et al. 1987.

Account had not been taken of the possible effect of short term peak exposures to benzene.

No account was taken of dermal exposure to benzene.

Tyre building work was carried out at one Pliofilm site with the possibility that benzene exposures had been higher there.

War production work during the period 1940-1945 is likely to have been associated with higher exposures and longer working hours.

It has also been suggested that the case control study may be compromised by poor matching of the exposure history of the control group compared to the cases (cases mean period exposed 8.9 years, controls mean period exposed 2.6 years) (CEFIC, 1988).

#### PLIOFILM RUBBER WORKERS

The vital status of a cohort of 1165 white males occupationally exposed for at least 1 day between January 1, 1940 and December 31, 1965 in the production of a natural rubber cast film at two localities in Ohio, was followed to December 31, 1981. The expected numbers of cause-specific deaths within 5-year age and 5-year calendar periods were based on death rates for the US white male population specific for the same 5-year age and calendar periods. At follow-up, 16 members of the cohort (1.4%) were unaccounted for but were assumed to be alive on December 31, 1981.

No statistically significant increases in total mortality (O/E=330/331.6; SMR=99.5) or deaths from malignant neoplasms (O/E=69/66.8; SMR=103) were found. Deaths from all lymphatic and haemopoietic neoplasms were significantly in excess (O/E=15/6.6; SMR=227; 95% CI=127-376); this being due mainly to excess numbers of leukaemia deaths (O/E=9/2.7; SMR=337; 95% CI=154-641) and multiple myeloma (O/E=4/1;

SMR=409; 95% CI=110-1047). The leukaemia deaths were of heterogeneous histological types (1 chronic myelogenous, 2 unspecified myelogenous and 6 acute myelogenous [ANLL]).

Industrial hygiene records gave background information on the atmospheric concentrations of benzene after 1946 and, for the most part, employees' 8-hr time-weighted average exposures appeared to be within the limits of the standard in effect at any given time (10-100 ppm). Occasional increases above these levels were reported, however, workers were required to wear respirators when the recommended limit was exceeded. The efficiency of these respirators was not reported.

The cumulative exposure for each cohort member was estimated from historical air-sampling data or from interpolation on the basis of existing data. The cohort was then divided according to levels of cumulative exposure; a level of 400 ppm-years equating to a mean annual exposure of 10 ppm over a 40-year working life. The SMRs for leukaemia deaths (SMRs=109, 322, 1186 and 6637) were found to markedly and progressively increase with a corresponding increase in cumulative exposure (1 ppm-day -39.99 ppm-years; 40-199.99 ppm-years; 200-399.99 ppm-years and >400 ppm-years respectively). These estimated cumulative exposure sub-groups contained 2, 2, 2 and 3 leukaemia deaths respectively. When halving or doubling these exposure ranges, the resultant SMRs continued to show a strongly positive trend of increasing risk with increasing exposure.

The time to death ranged from <5 to >30 years but no apparent pattern was evident.

A case-control analysis, conducted by comparing each cohort leukaemia death with ten controls matched for year of birth and first year of employment, indicated that the mean cumulative exposure was higher for the cases than the controls (254 and 50 ppm-years respectively), the average duration of exposure was longer (8.7 and 2.6 years respectively) and there was a difference in the rates of exposure (approximate averages of 24 and 16.5 ppm benzene/day respectively). It was suggested that the controls were selected from cohort members still alive at the time of death of the corresponding case.

The authors noted that, although hydrochloric acid, soda ash, natural rubber and small amounts of plasticizers were used, benzene was the only chemical in the plants that could reasonably be associated with haematologic toxicity (Rinsky et al. 1987). This study is an update of previous work (Infante et al. 1977; Rinsky et al. 1981).

**Source:**

Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (180) (221) (264) (558) (873) (938) (939) (1251)

**Remark:** PETROLEUM INDUSTRY WORKERS

A number of cohort studies have investigated oil refining and petroleum distribution workers but few have focussed specifically on benzene exposure.

A large cohort study of British refinery workers (34,781 workers employed for at least a year between 1950-1975) showed no excess mortality from leukaemia (Rushton & Alderson, 1981a). No benzene exposure measurements were made but when leukaemia cases and controls were qualitatively assigned to low, medium or high benzene exposure groups on the basis of job category in a nested case-control study, a significant association ( $p=0.05$ ) was reported between leukaemia and combined high or medium exposure compared to the low exposure group ( $RR=3.0$ , 95%  $CI=1.2-7.2$ ) (Rushton & Alderson, 1981b).

The 39 year follow up of this study has shown that in 34,569 refinery workers there was still no leukaemia excess (68 observed compared to 70 expected). In the follow up of the 23,306 distribution workers there were 61 leukaemias observed compared to 57 expected ( $SMR=108$ , 95%  $CI=83-140$ ) (Rushton, 1993).

The effects of occupational exposure to lower concentrations of benzene has been the subject of much study. The availability of data from further large cohorts occupationally exposed to benzene is relevant to defining the dose effect relationship at well characterized low exposures. Such data is likely to be forthcoming from the UK oil distribution cohort. This is currently the subject of a nested cohort study, due to report early in 1995.

Tsai et al. (1983) reported on 454 US refinery workers employed from 1952 until 1978 and found no deaths from leukaemia (0.4 expected). Personal samples indicated however that during the period 1973-1982 only 16% of 1394 samples indicated exposure to air concentrations (TWA) of >1 ppm.

Another US refinery study (retrospective follow-up study, 3976 white men) was apparently set up because of a suspected excess of leukaemia in the work force (McCraw et al. 1985). Fourteen leukaemia deaths were observed (6.6 expected) of which 8 were acute myeloid leukaemia (2.0 expected, significant increase).

14 cases of leukemia (8 acute myelogenous leukemia) occurred

among the work force of an oil refinery (8 cases expected); in a case-control study (50 matched controls) no particular job or department in this refinery was identified as the likely source of the excess leukemia deaths (Austin et al. 1986).

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (61) (750) (976) (978) (979) (1161)

**Remark:** UPDATE OF THE PLIOFILM COHORT STUDY  
method: study based on mortality data of a cohort of 1717 white male workers employed in pliofilm operations for at least 1 d and included 577 workers in jobs not considered by the studies of Rinsky et al. 1981 and 1987; mortality experience of the remaining 1140 workers was followed through 31 Dec. 1987  
results: significant excess of acute myelocytic or acute monocytic leukemia with strong dose-response trend; quadratic models predicted an additional lifetime risk of a benzene-related death from 45 years of exposure to 1 ppm of between 0.020-0.036 per thousand, linear models predicted risks of between 1.6 and 3.1 per thousand.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (265)

**Remark:** Hematological abnormalities in 217 workers exposed to benzene were compared to abnormalities in 100 hospital patients and personnel, and medical students of similar age.  
Workplace benzene concentrations ranged from 30-210 ppm, and length of exposure from 3 months to 17 years. Hematological abnormalities attributable to benzene (e.g., leucopenia, thrombocytopenia, pancytopenia, lymphocytopenia, giant platelets, eosinophilia, basophilia) were detected in 24% of the workers. No apparent association with duration of exposure was detected. About 1/3 of the workers were anemic, but when 1/3 of these anemic workers were treated with iron, the anemia disappeared so the anemia was not attributed to benzene alone. The authors conclude that the harmful effects of benzene are primarily on the leukocytes, with inconsistent findings of eosinophilia and basophilia, and thrombocytopenia and pancytopenia as secondary effects.

Repeated dose toxicity  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (18)

**Remark:** Repeated dose toxicity  
The authors studied 32 pancytopenic patients (n=30 males, n=2 females, age 15-57) who had been exposed to benzene concentrations ranging from 150 to 650 ppm for 4 months to 15 years. Analyses of a number of hematologic and bone marrow parameters were performed. Categorization of patients based on their bone marrow findings showed large variations in both the clinical and hematologic findings, ranging from asymptomatic to very severe. The authors conclude that a common basis in all cases is that benzene is the causative agent.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
German rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (19)

**Remark:** Repeated dose toxicity  
This short report summarizes the results from a cohort of shoe workers in Florence, Italy exposed to benzene between 1953-1964. Follow-up was through 1984. SMRs for males were 19.4 (66 observed) for aplastic anemia and 4.8 (6 observed) for leukemia. There was no apparent increase in mortality with increasing latency or duration of employment for either cause of death. Peak workroom exposure was in 1960 and averaged about 60 ppm, ~40 ppm from about 1953-1960, and dropping to zero between 1960 and 1964.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
German rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (864)

**Remark:** This study examined mortality among the Pliofilm cohort (n=1212 white male "wetside" workers) using an additional 6 years of follow-up (extended through December 31, 1987). Three different sets of job-, plant-, and year-specific exposure estimates were used in the analysis. The updated findings showed no additional cases of multiple myeloma; this does not support earlier Pliofilm reports of elevated mortality due to this disease. Extension of mortality follow-up did not substantially alter earlier estimates of leukemia risk associated with benzene exposure, and the choice of exposure estimated did not alter the conclusion. The results did, however, confirm the link between high dose benzene exposures and leukemia.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (877)

**Remark:** Using updated mortality information for the Pliofilm cohort (n=1868 Pliofilm workers from all departments), the authors performed proportional hazards modelling to estimate the dose-response for leukemia and occupational benzene exposure. Based on exposure estimates of Crump and Allen orof Paustenbach et al., 0.3-0.5 additional leukemia deaths per thousand workers with 45 ppm-years of cumulative benzeneexposure are predicted. These estimates are thought to be improved over earlier estimates by Rinsky et al., because proportional hazards modelling uses more of the available exposure data compared with the logistic modelling methods used by Rinsky et al.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (878)

**Remark:** This is a case-control study nested within a cohort of men employed at 8 oil refineries in the U.K. Thirty-six leukemia cases were matched with two sets of 108 controls. The matching criteria included refinery and year of birth inone set, and in the second set of controls, length of service was an added criterion. Exposure to benzene was based on job history and qualitative ranking of low, medium, and high. The risk for men with medium/high benzene exposure was about twice that of the risk of men with low exposure. The authors conclude that if there is an increased risk of leukemia due to benzene exposure in oil refineries, only a very small proportion of workers could beaffected.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment  
07-JUL-2005 (977)

**Remark:** Results of an extension of follow up (1976 to 1989) of a cohort of workers employed for at least one year between 1 January 1950 and 31 December 1975 at oil distribution centers in Britain are presented. Over 99% of the workers were successfully traced to determine their vital status at 31 December 1989. The mortality observed was compared with that expected from the death rates of all the male population of England and Wales. The mortality from all causes of death for the total study population was less thanthat of the comparison population, and reduced mortality wasalso found for many of the major non-malignant causes of death. No healthy worker effect was found for ischaemic heart disease, and raised mortality from this disease was found in particular for one company and in several job groups. Raised mortality was also found for aortic aneurysm. Mortality from all neoplasms was lower than expected overall, largely due to a deficit of deaths from

malignant neoplasm of the lung. Raised mortality patterns from all neoplasms, malignant neoplasm of the lung, and several non-malignant disease groups were found for general manual workers although the mortality from many of these diseases for all men in this social class in the national population is also high. There was increased mortality from malignant neoplasms of the larynx and prostate but these

tended to be in isolated subgroups. Mortality from malignant neoplasm of the kidney was raised overall and in drivers in particular. Mortality from leukaemia was high at one company and in drivers overall.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(980)

**Remark:**

A retrospective mortality study was conducted among 6672 petroleum marketing and distribution workers from 226 locations throughout Canada. These employees worked for at least one year in the marketing distribution segment from 1964 through 1983 or were annuitants as of 1964. Industrial hygienists assigned hydrocarbon (HC) exposure frequency scores for several jobs, departments, and job functions. Dermal and inhalation exposures were considered.

Standardized mortality ratios were calculated for the total cohort, HC exposure frequency groups, and tank truck drivers, and Poisson regression techniques were used to model mortality for selected causes of death according to HC exposure frequency. Results indicated overall mortality below that of the general Canadian population for all marketing distribution workers [Standardized mortality ratio (SMR) = 0.88]. Tank truck drivers showed significantly elevated mortality due to leukemia (SMR = 3.35) based on five deaths. The leukemia findings were not evident in the larger group of marketing distribution workers classified as exposed to hydrocarbons (SMR = 1.01). No other cause of death was elevated in truck drivers. The leukemia findings are suggestive of a possible influence due to exposure to HCs in tank truck drivers, although other explanations cannot be ruled out. Other findings of elevated mortality in the marketing distribution group are generally not statistically significant. These included moderately increased mortality due to multiple myeloma, malignant melanoma, and kidney cancer. Small numbers of observed and expected deaths limit concise interpretations for these diseases.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1021)

**Remark:** The authors examined leukemia risk by quantitative benzene exposure among petroleum distribution workers using a case-control study design. There were 14 leukaemia cases and 55 controls who were part of a previously studied population of Canadian petroleum distribution workers (Schnatter et al., 1993). Exposure to benzene occurred mostly in petrol truck drivers, loaders, and terminal operators. In this setting, benzene exposure derives from a hydrocarbon mixture, which also contains toluene, xylene, etc. However, inhibition of benzene's effects by other hydrocarbons has not been shown to occur for the low levels of benzene included in this study. Exposures over a working career averaged from 0.3 to 19.8 mg/m<sup>3</sup> (.01 to 6.2 ppm), although a substantial fraction of cases and controls experienced a long term average concentration below 3.2 mg/m<sup>3</sup> (1 ppm) over their career. Cumulative exposure ranged from less than 1 to 220 ppm-years. The risk [measured in terms of an Odds Ratio (OR)] for all leukaemias was calculated as 1.002 per ppm-year in a logistic regression model. Several categorizations of exposure were made, but in no case was there a monotonic dose-response relationship. However, the study was relatively small.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005 (1024)

**Remark:** This is an update of a subgroup of the population involved in the Wong (1987) study of chemical manufacturing workers.

The study includes 4172 men hired between 1940 and 1977 in a plant which used benzene in five processes: nitrobenzene, phenol production, chlorobenzene, muriatic acid, and alkylbenzenes. Exposures occurred in maintenance and production employees, but long term average (LTA) concentrations were estimated only for the 666 production workers ever exposed to benzene. Cumulative exposures ranged from 0.01 to 632 ppm-years in these workers. The number of short term exposure episodes greater than 320 mg/m<sup>3</sup> (100 ppm) (exposures predominantly for 15 minutes) also was estimated for all (i.e. maintenance and production) workers. The study reported one AML versus 0.27 expected for the < 1 ppm-year category, no AML's (versus 0.23 expected) in the 1-6 ppm-year category, and one AML in the 6+ ppm-year category (versus 0.22 expected). Two CLL's showed exactly the same pattern, although the expected numbers were slightly lower. For all leukaemia types combined, there were three observed versus 0.65 expected cases in the 6+ ppm-year category [SMR = 4.6, 95% CI = (0.9, 13.4)]. When the results are analyzed in terms of the number of 15 minute episodes of exposure to short term levels which exceeded 100 ppm (referred to as "peaks" in

thereport), a statistically significant excess of all leukaemias was found for employees experiencing the highest number (more than 40) of peaks. There were 5 total leukaemias versus 1.25 expected [SMR = 4.0, 95% CI = (1.3, 9.2)]. The SMR for AML in these workers was slightly higher(4.8 based on two cases), but was not statistically significant.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(565)

**Remark:**

In the present investigation, all cohort studies of petroleum workers in the United States and the United Kingdom were combined into a single database for cell-type-specific leukemia analysis. The majority of these workers were petroleum refinery employees, but production, pipeline, and distribution workers in the petroleum industry were also included. The combined cohort consisted of 208,741 petroleum workers employed between 1937 and 1989. Cell-type-specific leukemia risks were calculated using a meta-analysis procedure appropriate for combining occupational cohort studies. These risks were expressed in terms of cell-type-specific leukemia standardized mortality ratios (meta-SMRs). The meta-SMR for acute myelogenous leukemia was 0.96. The lack of an increase of acute myelogenous leukemia was attributed to the low levels of benzene exposure in the petroleum industry, particularly in comparison to benzene exposure levels in some previous studies of workers in other industries, who had been found to experience increased risk of acute myelogenous leukemia (AML). Using the Rinsky et al. (1987) data from the Pliofilm cohort, exposure-response analyses for AML indicated no increase for cumulative exposures below 200 ppm-years (SMR = 0.91). Had other more realistic higher exposure estimates been used, the observed AML threshold would have been higher. No increase in chronic myelogenous, acute lymphocytic, or chronic lymphocytic leukemias was found in petroleum workers (meta-SMRs of 0.89, 1.16, and 0.84, respectively). Meta-analyses restricted to refinery studies or to studies with at least 15 years of follow-up yielded similar results. The findings of the present investigation are consistent with those from several recent case-control studies.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1277)

**Remark:**

A retrospective cohort study was carried out in 1982-1983 among 28,460 benzene-exposed workers (15,643 males, 12,817 females) from 233 factories and 28,257 control workers (16,621 males, 12,366 females) from 83 factories in 12 large cities in China. All-cause mortality was significantly higher among the exposed (265.46/100,000 person-years) than among the unexposed (139.06/100,000 person-years), as was mortality from all malignant neoplasms (123.21/100,000 versus 54.7/100,000, respectively). For certain cancers, increased mortality was noted among benzene-exposed males in comparison with that among unexposed males; the standardized mortality ratios (SMR) were elevated for leukemia (SMR = 5.74), lung cancer (SMR = 2.31), primary hepatocarcinoma (SMR = 1.12), and stomach cancer (SMR = 1.22). However, no analyses by duration of benzene exposure were performed for lung cancer, primary hepatocarcinoma, or stomach cancer to determine their relationship with benzene.

For females, only leukemia occurred in excess among the exposed. Risk of leukemia rose as duration of exposure to benzene increased up to 15 years, and then declined with additional years of exposure. Leukemia occurred among some workers with as little as 6 to 10 ppm average exposure and 50 ppm-years (or possibly less) cumulative lifetime exposure (based on all available measurements for the exposed work units). Among the 30 leukemia cases identified in the exposed cohort, the proportion of subjects with acute lymphocytic leukemia was substantially lower and the proportion with acute nonlymphocytic leukemias was higher in the general population. During 1972 to 1981, the annual incidence of leukemia ranged from 5.83 to 28.33 per 100,000 with higher rates occurring in the interval 1977 to 1981 than in the earlier years of the study period.

**Source:**

Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:**

Risk Assessment

07-JUL-2005

(1300)

**Remark:**

This is a case-control study of leukemia in relation to quantitative estimates of benzene exposure among petroleum distribution workers. There were 90 leukaemia cases and 354 controls from a previously studied United Kingdom petroleum worker population (Rushton, 1993). Exposure assessment was modelled after the Canadian effort (Armstrong et al., 1996). Leukaemia cell type information was obtained through death certificates and a cancer registration system.

Benzene exposure concentrations were similar to the Canadian study, though slightly lower. Work history information was not as complete as the Canadian study, and

incomplete or missing personnel records had to be supplemented with medical records, interviews, and inferred interpolations (based on patterns observed in complete work histories). Rather than deleting workers with incomplete work histories from the study, the investigators performed sensitivity analyses to examine the effect of poorly documented work histories on the results. For total leukaemias, the logistic model produced an OR of 1.004 per ppm-year. Risk was greater for duration of employment (OR = 1.03 per year) rather than average exposure concentration (OR = 0.97 per ppm). When cumulative exposure was broken up into quintiles, OR's ranged from 1.3 to 2.6, but the highest OR was in second quintile, and no OR was statistically significant.

For AMML, using continuous benzene exposure measures, with no categorization and no resultant loss of information inherent in the categorization, there was no relationship between AMML and cumulative benzene exposure, average exposure concentration over a career [long-term average - work history (LTA-WH)], nor the long term average concentration in the maximally exposed job of a worker's career (LTA-MEJ). There was a slight suggestion of a risk for the simple measure of duration (years) of exposure, but this was not statistically significant. When exposure metrics were categorized, the non-linear exponential dose-response relationship inherent in the continuous analysis is no longer assumed. The dose-response relationships with categorical classifications into discrete ranges show a varying pattern dependent on the metric chosen.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(981)

**Remark:** Many risk assessments for leukemia associated with benzene exposure have been based on the mortality experience of the rubber hydrochloride worker cohort. Although there have been several different historical exposure assessments proposed for this cohort, Paustenbach et al. [1992, *J Tox Environ Health*], recently published a new historical characterization of benzene exposures based on data previously developed by Rinsky et al. [1981, *Am J Ind Med*] and further modified by Crump and Allen [1984: OSHA]. Adjustments by Paustenbach et al. in the Rinsky et al. data result in retrospective benzene exposure estimates far greater than those previously reported, by an order of magnitude in many cases. Judgments made on the significance of dermal contact and interpretation of historical measurement data led Paustenbach et al. to arrive at exposure estimates for this cohort that are in conflict

with what is known about the adverse effects of benzene exposure. More reasonable estimates for dermal absorption are included in this report that do not substantially affect total estimates of benzene exposure for the cohort. The exposure estimates originally presented in the Rinsky et al. article appear in concordance with data previously reported in any analyses.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1193)

**Remark:**

Lymphohaematopoietic malignancies and quantitative estimates of exposure to benzene in Canadian petroleum distribution workers.

OBJECTIVE: To evaluate the relation between mortality from lymphohaematopoietic cancer and long term, low level exposures to benzene among male petroleum distribution workers. METHODS: This nested case control study identified all fatal cases of lymphohaematopoietic cancer among a previously studied cohort. Of the 29 cases, 14 had leukemia, seven multiple myeloma, and eight non-Hodgkin's lymphoma. A four to one matching ratio was used to select a stratified sample of controls from the same cohort, controlling for year of birth and time at risk. Industrial hygienists estimated workplace exposures for benzene and total hydrocarbons, without knowledge of case or control status, for combinations of job, location, and era represented in all work histories. Average daily benzene concentrations ranged from 0.01 to 6.2 parts per million (ppm) for all jobs. Company medical records were used to abstract information on other potential confounders such as cigarette smoking, although the data were incomplete. Odds ratios (ORs) were calculated with conditional logistic regression techniques for several exposure variables. RESULTS: Risks of leukaemia, non-Hodgkin's lymphoma, and multiple myeloma were not associated with increasing cumulative exposure to benzene or total hydrocarbons. For leukaemia, the logistic regression model predicted an OR of 1.002 ( $P < 0.77$ ) for each ppm-y of exposure to benzene. Duration of exposure to benzene was more closely associated with risk of leukaemia than other exposure variables. It was not possible to completely control for other risk factors, although there was suggestive evidence that smoking and a family history of cancer may have played a part in the risk of leukaemia. CONCLUSION: This study did not show a relation between lymphohaematopoietic cancer and long term, low level exposures to benzene. The power of the study to detect low-such as twofold-risks was limited. Thus, further study

on exposures to benzene in this concentration range are warranted.

**Source:** German Rapporteur  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
07-JUL-2005 (1020)

**Remark:** An excess of lympho-haematopoietic (LH) cancers has been identified in the Australian petroleum industry through the Health Watch surveillance programme. A nested case-control study is being conducted to investigate this excess. This paper describes the methods used to provide quantitative estimates of benzene exposure for each of the subjects in the case-control study. Job histories were compiled for each subject from interviews and company employment records. Site visits and telephone interviews were used to identify the tasks included in each job title. Details about the tasks such as their frequency, the technology in use and about changes that had taken place over the years were also gathered. Exposure dated back to the late 1940s for a few subjects. Collaborating petroleum companies provided recent benzene exposure monitoring data. These were used to generate Base Estimates of exposure for each task, augmented with data from the literature where necessary. Past exposures were estimated from the Base Estimates by means of an exposure algorithm. The modifying effects of technological changes and changes to the product were used in the algorithm. The algorithm was then computed to give, for each job, for each subject, an estimate of average benzene exposure in ppm in the workplace atmosphere (Workplace Estimate). This value was multiplied by the years for which the job was held and these values summed to give an estimate of Cumulative Estimate of benzene in ppm-years. The occupational hygienists performing the exposure assessment did so without knowledge of the case or control status of subjects. Overall exposures to benzene in the Australian petroleum industry were low, and virtually all activities and jobs were below a time-weighted average of 5ppm. Exposures in terminals were generally higher than at refineries. Exposures in upstream areas were extremely low. Estimates of Cumulative Estimate to benzene ranged from 0.005 to 50.9 ppm-years. Retrospective exposure assessment for benzene in the Australian petroleum industry.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
German Rapporteur  
**Reliability:** (1) valid without restriction  
**Flag:** Risk Assessment  
07-JUL-2005 (414) (495)

**Remark:** The concentrations of benzene, toluene, ethylbenzene, and o-,m- and p-xylene were measured in venous blood samples collected from 13 non-smokers and 14 cigarette smokers. The subjects had no known occupational or hobby-related exposure. The blood samples were analysed by a purge and trap technique followed by gas chromatography/mass spectrometry/computer analysis. The above-mentioned volatile organic compounds (VOC) could be detected in measurable amounts in all blood samples. This finding seems to reflect the ubiquitous exposure of humans to these agents in the urban environment. Smokers were found to have significantly higher blood concentrations of benzene (median 493 ng/l) and toluene (median 2001 ng/l) than non-smokers (median 190 ng/l and 1141 ng/l, respectively). The detection limits in whole blood were: benzene, 21 ng/l; toluene, 80 ng/l. The results indicate that smoking is associated with a significant additional exposure to VOC, in particular to benzene and toluene.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (467)

**Remark:** To obtain reference values for the biological monitoring of benzene, the kinetics of benzene (1.6 or 9.4 ppm, 4-h-exposure) were studied in three healthy nonsmoking volunteers. The amount of benzene absorbed into the body was estimated from the average difference in the concentration of inhaled and exhaled air. It was 48 % for the high exposure and 52 % for the low exposure. The elimination of benzene after the high exposure showed a good fit to a three-compartment model. The mean half-times for benzene were 55 and 61 min for the alpha phase, 3.2 and 5.9 h for the beta phase and 19.7 and 14 h for the gamma phase for blood and expired air, respectively. The level of phenol in urine increased during the 4 h of exposure from the starting level of 26-74 micromol/l to a maximum of 192-315 micromol/l. The excretion of phenol returned to the level found before the exposure in about 14-35 h.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (880)

**Remark:** In one autopsy report performed on a 18 year old male youth who died while sniffing reagent grade benzene, the following benzene concentrations were reported: 3.9 mg% in brain, 2.23 mg% in abdominal fat, 2.0 mg% in blood, 1.9 mg% in kidney,

1.6 mg% in liver, 1.1 mg% in bile, 1.0 mg% in stomach and 0.06 mg% in urine.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (1260)

**Remark:** Repeated dose toxicity  
Report on 200 chemical workers and 268 controls exposed to benzene levels between 0.032 and 4.48 mg/m<sup>3</sup> (0.01 and 1.4 ppm). No differences in RBC, WBC, Hb, platelets, or MCV were found when benzene was treated as a continuous exposure. A small difference in MCV was found for "current" exposure. Several confounders were controlled, and it was shown that smoking had significant effects on most studied parameters. The authors judged that since the MCV effect was only 0.4%, it was not of clinical significance.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (226)

**Remark:** In this report the Commission of the European Communities (CEC 1993) notes on the preliminary results of an unpublished study (Van Damme et al. 1991).  
Repeated dose toxicity

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (178)

**Remark:** Repeated dose toxicity  
Review on benzene exposure at workplaces

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (233)

**Remark:** Estimations by Hoffmann et al (1990/1991) stated that a smoker absorbs about 400 µg benzene when smoking 20 cigarettes/day.  
Repeated dose toxicity

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (518)

**Remark:** In this study, workers were exposed to benzene concentrations ranging from 110-160 mg/m<sup>3</sup> (34-50 ppm) in 1971, and 11-22 mg/m<sup>3</sup> (3 to 7 ppm) in 1972. No reason for the decrease in exposure was given. Several blood tests revealed abnormalities in women including lowered RBC counts (3.8 x 10<sup>6</sup>/mm<sup>3</sup>) and elevated MCV (105 fL). In men, who were in general more highly exposed, the values were 4.1 x 10<sup>6</sup> cells/mm<sup>3</sup> and 103 fL, respectively. Thrombocytopenia and

elevated alkaline phosphatase were also noted. This study is not sufficient for defining a NOAEL or LOAEL since it is not possible to conclude which exposure levels were responsible for the effect. Also, there were potentially confounding exposures to other substances in these workers.

Repeated dose toxicity  
**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (662)

**Remark:** McNeal et al. (1993) found experimentally that benzene in food and beverages is present at levels equal or less than 2 ng/g (see Appendix II Table 15).

Repeated dose toxicity  
**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (757)

**Remark:** Repeated dose toxicity  
Report of benzene in fish caught in Japanese sea water near refineries.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (855)

**Remark:** Repeated dose toxicity  
The population studied by Kipen (1988/89) is part of the study reported by Rinsky et al. (1987), often referred to as the Pliofilm<sub>2</sub> cohort. A job exposure matrix consisting of 493 job versus time period entries was constructed, 86 (17%) of which were filled with actual exposure monitoring information. All entries prior to 1947 (i.e., from 1939) were done through extrapolation of more recent measurements.  
In the 1989 report, Kipen et al. used two exposure extrapolation schemes (A and B) and related each to blood counts which included RBC, WBC and Hb. To obtain reliable trends for each worker, the authors analyzed those with more than five blood tests (264 workers and 16841 tests). The authors reported an increase in blood counts in the 1940's, and a leveling off thereafter. When using extrapolation scheme B, Kipen et al. (1989) found a strong correlation between benzene exposures between 112-438 mg/m<sup>3</sup> (35-137 ppm) [mean: 240 mg/m<sup>3</sup> (75 ppm)] and decreased WBC counts (between 1940-48). There also was a strong correlation for decreased RBC counts, but the correlation with Hb values was lower.

For 1949-75, when the mean exposure (long-term average concentrations) was about 48-64 mg/m<sup>3</sup> (15-20 ppm), no such correlation could be observed with any of the three haematology measures. Extrapolation scheme A, which predicted lower exposures, particularly in 1940-48, did not correlate with decreases in WBC, RBC, nor Hb in either time period. This scheme predicted exposures from 19.2 to 60.8 mg/m<sup>3</sup> (6 to 19 ppm) in 1940-48. In either case (extrapolation scheme A or B), the data suggest that exposures of 64 mg/m<sup>3</sup> (20 ppm) or less have no effect on WBC, RBC,

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (941)

**Remark:** Repeated dose toxicity  
Study suggests RBC reduction may not be the most sensitive effect of benzene haematotoxicity.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (1006)

**Remark:** Presentation of health examination findings in a subset of 282 men studies of a cohort study of mortality in 594 occupationally exposed individuals. Although slight decreases on RBC values were detected, these findings were not regarded as clinically significant. Benzene No correlations were found between peripheral blood counts and latency, duration or intensity of benzene exposure. Benzene levels ranged between <1 - 35 ppm (TWA). Repeated dose toxicity

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (1150)

**Remark:** A cohort of 74 828 benzene exposed and 35 805 unexposed workers employed from 1972 through 1987 in 632 factories in 12 cities in China was identified and followed up to determine the incidence of haematological neoplasms and related disorders. Estimates of benzene exposure were derived from work histories and available historic benzene measurements. The large number of workers, factories and cities provides a high diversity of environmental scenarios. For workers historically exposed to benzene at average levels of less than 10 ppm the RR for haematological neoplasms combined was 2.2 (95% confidence interval (CI)=1.1-4.2), and, for the combination of acute nonlymphatic leukaemia (ANLL) and related myelodysplastic syndromes (MDS), the RR was 3.2 (95% CI=1.0-10.1). For individuals who were occupationally exposed to benzene at constant levels of 25 ppm or more, the RR for the combination of acute nonlymphatic leukaemia and related

myelodysplastic syndrom was 7.1 (95% CI=2,1-23.7). Workers with 10 or more years of benzene exposure had an RR of developing non-Hodgkin's lymphoma of 4.2 (95% CI=1.1-15.9), and the development of this neoplasm was linked most strongly to exposure that had occurred at least 10 years before diagnosis. The findings suggest, that recent benzene exposure is predictive of subsequent risk for ANLL/MDS. In contrast, recent exposure was only weakly linked to non-Hodgkin's lymphoma.

**Source:** Cohort study (China)  
**Flag:** German rapporteur  
Risk Assessment  
07-JUL-2005 (486)

**Remark:** Chromosome analysis from peripheral blood lymphocytes of workers after an acute exposure to benzene.  
test system: chromosomal aberrations and sister-chromatid exchanges in peripheral lymphocytes

no. of subjects: 10

expos. concentr.: not given

**Source:** authors' conclusion: negative  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (214)

**Remark:** Cohort Study (USA)  
Ott performed a historical cohort study (Michigan Division Dow Chemical) on 594 individuals. In the total study sample, significantly more cases (n = 3) of acute non-lymphatic leukaemia (ANLL) were observed as compared to 0.8 expected cases (p = 0.047). However, if individuals who had been exposed to other chemicals (e.g. arsenic, asbestos) were excluded from the calculation of the Standardized Mortality Ratios (SMR) seems to be unclear; neither the total mortality nor the disease-specific mortality were increased.

**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (863)

**Remark:** Chromosome changes in patients with chronic benzene poisoning.  
test system: chromosomal aberrations and aneuploidy in peripheral lymphocytes

expos. concentr.: not given (subjects suffered from chronic benzene poisoning)

no. of subjects: 21

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (299)

**Remark:** Cytogenetic studies in thirteen patients with pancytopenia and leukemia associated with long-term exposure to benzene.  
test system: chromosomal aberrations and aneuploidy in peripheral lymphocytes

expos. concentr.: 150 - 210 ppm

no. of subjects: 13

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (336)

**Remark:** Chromosome studies in workers exposed to benzene or toluene or both.  
test system: chromosomal aberrations in peripheral lymphocytes

expos. concentr.: not given (subjects suffered from chronic benzene poisoning)

no. of subjects: 25

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (364)

**Remark:** Chromosome changes and their evolution in subjects with past exposure to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes

expos. concentr.: 125-532 ppm

no. of subjects: 10

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (366)

- Remark:** Chromosome studies in workers exposed to benzene. Berg K. (Eds.), Genetic damage in man caused by environmental agents.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: 0.08 - 10 ppm (4 groups)  
no. of subjects: 9 to 12 per group  
authors' conclusion: inconclusive
- Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (372)
- Remark:** Gasoline pump mechanics had increased frequencies and sizes of micronuclei in lymphocytes stimulated by pokeweed mitogen.  
test system: micronuclei in peripheral lymphocytes  
expos. concentr.: 0.31 ppm (peaks 6.2 ppm)  
no. of subjects: 15  
authors' conclusion: positive
- Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (513)
- Remark:** Cytogenetic analysis of peripheral blood lymphocytes in workers exposed to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: 0.45 - 11.25 ppm  
no. of subjects: 66  
authors' conclusion: negative
- Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (576)
- Remark:** Cytogenetic monitoring of industrial populations potentially exposed to genotoxic chemicals and of control populations.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: <0.09 ppm  
no. of subjects: 32

**Source:** authors' conclusion: negative  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (586)

**Remark:** Possible Genotoxicity in low level benzene exposure.  
test system: chromosomal aberrations and  
sister-chromatid exchanges in peripheral lymphocytes

expos. concentr.: 8 ppm (group I), 5 ppm (group II)

no. of subjects: group I, 38; group II, 45

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (599)

**Remark:** test system: micronuclei and 8-hydroxy-deoxyguanine in  
peripheral lymphocytes

expos. concentr.: I, <12 ppm; II, 12-60 ppm ; III,  
>60 ppm

no. of subjects: I, 35; II, 24; III, 28

authors' conclusion: positive for both endpoints  
The study of DNA oxidative damage in benzene-exposed  
workers.

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (696)

**Remark:** Chromosome aberration, sister-chromatid exchange,  
proliferative rate index, and serum thiocyanate  
concentration in smokers exposed to low-dose benzene  
test system: chromosomal aberrations and  
sister-chromatid exchanges in peripheral lymphocytes

expos. concentr.: 2.2 ppm

no. of subjects: 42

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (730)

**Remark:** Genotoxic effects in workers exposed to low levels of  
benzene from gasoline.  
test system: DNA single strand breaks and urinary  
8-hydroxy-deoxyguanine levels

expos. concentr.: 0.13 ppm  
no. of subjects: 33  
authors' conclusion: positive  
**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (831)

**Remark:** Cytogenetic study of workers exposed to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: 2.1 ppm  
no. of subjects: 52  
authors' conclusion: positive  
**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (892)

**Remark:** Investigations of the frequency of DNA strand breakage and cross-linking and of sister chromatid exchange frequency in the lymphocytes of female workers exposed to benzene and toluene.  
test system: DNA single strand breaks and sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: 1.3 ppm  
no. of subjects: 20  
authors' conclusion: positive  
**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (901)

**Remark:** Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans.  
test system: glycophorin A mutation assay in bone marrow cells  
exposure concentr.: 72.2 ppm (2.4 - 301.1 ppm)  
no. of subjects: 24  
authors' conclusion: positive  
**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (963)

**Remark:** A cytogenetic study on workers exposed to low concentrations of benzene.  
test system: chromosomal aberrations and sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: 0.2 - 12.4 ppm  
no. of subjects: 22  
authors' conclusion: positive for aberrations, negative for sister-chromatid exchanges

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (999)

**Remark:** Localization of breakpoints in the karyotype of workers professionally exposed to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: <31 ppm  
no. of subjects: 33  
authors' conclusion: positive

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1001)

**Remark:** Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: < 10 ppm  
no. of subjects: 56  
authors' conclusion: positive

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1000)

**Remark:** Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. Int.  
test system: sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: 50 ppm  
no. of subjects: 36

**Source:** authors' conclusion: negative  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1029)

**Remark:** Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in frequencies of chromosomal aberrations in peripheral blood lymphocytes.  
test system: chromosomal aberrations and sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: 0.9 to 21.5 ppm  
no. of subjects: 3 groups of 10, 22, 17 subjects

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1144)

**Remark:** Chromosome studies on workers exposed to atmospheric benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: I: 25-150; II: 25-150; III: 12 ppm  
no. of subjects: I, 20; II, 12; III, 20

**Source:** authors' conclusion: I, positive; II and III, negative  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1148)

**Remark:** Analysis of chromosomal aberrations in shoe workers exposed long term to benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: not given  
no. of subjects: 58

**Source:** authors' conclusion: positive  
German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1174)

**Remark:** Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers.  
test system: chromosomal aberrations and sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: I, 3-50 ppm; II, 0-9 ppm  
no. of subjects: I, 7; II, 9  
authors' conclusion: negative

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1240)

**Remark:** Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene.  
test system: sister-chromatid exchanges in peripheral lymphocytes  
expos. concentr.: <1 - 10 ppm  
no. of subjects: 66  
authors' conclusion: negative

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1294)

**Remark:** Analysis of chromosomal aberrations in workers exposed to low level benzene.  
test system: chromosomal aberrations in peripheral lymphocytes  
expos. concentr.: <1 - 10 ppm  
no. of subjects: 48  
authors' conclusion: inconclusive

**Source:** German rapporteur (813)  
**Flag:** Risk Assessment  
07-JUL-2005 (1295)

**Type of experience:** other: biological monitoring

**Remark:** Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene, and there is some evidence that urinary phenol levels can be correlated with exposure level. However, correlating urinary phenol with benzene exposure is complicated by potentially high and variable background levels of phenol that results from ingestion of vegetables, exposure to other aromatic compounds, ingestion of ethanol, and inhalation of cigarette smoke.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (560)

**Type of experience:** other: biological monitoring

**Remark:** Benzene-metabolite-DNA-adducts were observed in the bone marrow of rats at doses that did not affect bone marrow cellularity and the authors suggested that monitoring of these DNA-adducts might be a sensitive bioassay of genotoxic effects of benzene exposure.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (652)

**Type of experience:** other: biological monitoring

**Remark:** A correlation of benzene concentrations in air and blood with regard to urinary concentrations of muconic acid and S-phenyl-N-acetyl cysteine were observed in car mechanics.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (900)

**Result:** Case-control studies in sub-collectives from the studies described by Yin et al (1996) in rubber industry, manufacturing of adhesive tapes, and paint-and varnish factories were performed by Rothman et al. (1996a, 1996b) and Dosemeci et al. (1997). In these studies workers exposed to benzene were compared to a cohort of non-exposed individuals who had no history of benzene exposure and other marrow-toxic chemicals, or ionizing radiation. They were frequency-matched to the exposed subjects on 5 year age intervals and gender. The exposure level was 1 - 328 ppm (Rothman et al., 1996a) for a period of at least six months. There was a significant difference between cases and controls in the following parameters: WBC, lymphocytes, platelet and RBC counts were significantly reduced. APA somatic cell mutation frequency was about as twice as high in the benzene exposed individuals as compared to controls. Sub-collectives of these cohorts were further studied: In a group of persons that were exposed to levels below 31 ppm of benzene (range 1 - 30.6 ppm, median 13.6 ppm), a significant decrease of WBC, lymphocyte and platelet counts remained, and a further group of persons exposed to 1 - 20 ppm, median 7.6 ppm showed decreased lymphocyte count. There was an increase in the relative risk for benzene induced health impairment from 1 to 2.2 ppm in persons exposed to < 5 ppm compared to 5-19 ppm. A NOAEL for that effect that can be deduced from these studies is 1 ppm.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (303) (967) (968)

**Result:** The relation between lympho-haematopoietic cancer and exposure to benzene was subject of a subsequent nested case-control study. The case-control study included 79 cases of lympho-haematopoietic cancers, 33 leukemias, 31 non-Hodgkin's lymphomas, and 15 multiple myelomas. The leukemias consisted of 9 AML, 6 CML, 2 ALL, 11 CLL, and 5 other types. Individual exposure estimates were established based on 18 different job-groups. The exposure estimates in this study are probably of high reliability since they are based on more actual measurements than previous occupational studies on the leukomogenic action of benzene. Relations between cancers and various types of exposure metrics were established (cumulative exposure, exposure duration, exposure intensity, start date of exposure, influence of "peak" exposures). Lifetime cumulative exposures were low for the majority of subjects (0.005 - 57.3 ppm-years, mean 4.9 ppm-years). Nearly 85% of the subjects had an exposure < 10 ppm-years while only 3.6% had an exposure > 40 ppm-years. Average exposure intensity was less than 1 ppm for 98% of the individuals (range 0.001 to 2.07 ppm). The total incidence of lympho-haematopoietic cancers was strongly associated with total benzene exposure. The exposure group = 8 ppm-years had a mean OR of 3.32. The strongest association was found with exposure between 5 to 15 years prior to diagnosis. Recent exposure within 5 years made only a small contribution. There was no association with the duration of employment nor with the starting date of employment. A very strong association was found for leukemias alone, at exposure = 16 ppm-years an OR was found of about 35. No association was found with employment duration and start period of employment. A strong association was observed for leukemia subtypes AML (or ANLL) and CLL. No associations were found for chronic myeloid leukemia or other leukemia types (nor NHL and multiple myeloma).

**Source:** German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(789)

**Result:** There are data that benzene may be present as a contaminant in consumer products. According to these data paints may contain a maximum content of 390 ppm (= 1.24 mg/l), lubricants and adhesives of 410 ppm (=1.3 mg/l), and model- and hobby glues of 780 ppm (=2.48 mg/l). From these data as a worst case assumption the maximum probable content of benzene in consumer products of 2.5 mg/l may be derived.

**Source:** German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(927)

**Result:** Wallace & Pellizari (1986) have measured benzene air concentrations in homes of smokers and non-smokers. During fall and winter, mean benzene air levels were 16 and 9.2  $\mu\text{g}/\text{m}^3$  in homes of smokers and non-smokers, respectively. The difference between both air values (about 7  $\mu\text{g}/\text{m}^3$ ) can be considered as contribution of smoking to the air concentration of benzene in a non-smoker house. Thus, this concentration resulting from smoking has been taken into account for exposure estimates for non-smoking people (passive smokers). In spring and summer, the respective concentrations were 4.8  $\mu\text{g}/\text{m}^3$  (smokers) and 4.4  $\mu\text{g}/\text{m}^3$  (non-smokers). The authors do not specify the variation of the data. For passive smokers, it is assumed that exposure duration is 24 hours (worst case) as a non-smoking family member.

**Source:** German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(1226)

**Result:** The estimate of benzene exposure by passive smoking is in agreement with the uptake of 30  $\mu\text{g}$  per day which has been given by Eikmann et al. (2000). However, taking into consideration, that the air concentrations measured by Wallace & Pellizari (1986) are mean values, these estimates do not characterize extremes of exposures. A comparison with concentrations of benzene in blood of smokers shows high variability with a range from < 60 up to 950 ng/l (95th percentile: 850 ng/ml). It must therefore be assumed, that exposure to benzene may exceed the estimated values considerably.

**Source:** German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(326)

**Result:** It is reported by CONCAWE (2000) that at filling stations the average benzene concentration during a three minutes car refuelling is 0.8-1.0  $\text{mg}/\text{m}^3$ . According to exposure data during gasoline refuelling the overall arithmetic mean concentration for benzene in the integrated samples ( $n = 8$ ) were 0.90  $\text{mg}/\text{m}^3$  with gasoline containing less than 1% benzene.

**Source:** German Rapporteur

**Flag:** Risk Assessment

07-JUL-2005

(234)

**Result:** A data set consisted of 8 integrated samples covering each approximately 20 refuellings for a total of 167 operations (Vainiotalo et al., 1999; addendum September 11, 2002). For risk characterisation the maximum value of this data set of 1.3  $\text{mg}/\text{m}^3$  will be selected. Higher concentrations of 4.3  $\text{mg}/\text{m}^3$  have been given by Vainiotalo et al. (1999) from a previous study during 1984-1985 in which the average benzene content of gasoline was reported to be 4% (w/w).

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (1195)

**Result:** The benzene concentration declines within a period of 10 weeks to less than 10% of this concentration (Brown and Cheng, 2002) thus an air concentration of 12 µg/m<sup>3</sup> can be assumed as worst case exposure. Driving an old car, the internal concentrations will be even lower. Benzene exposure will increase considerably if the driving duration is increased extensively.

**Source:** German Rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (156)

**Remark:** Midzenski showed that even short exposure to benzene in 15 degassers of 5 days up to 3 weeks may possibly lead to haematological alterations. 2 workers who were exposed to benzene with concentrations above 192 mg/m<sup>3</sup> (60 ppm) showed WBC counts below 4.500/mm<sup>3</sup> and 5 workers Hb values below normal two days after exposure (Midzenski et al. 1992). A control group was not examined. One month after exposure, 6 of the 15 workers had large granular lymphocytes in blood smears. Blood abnormalities did not correlate with either duration or frequency of exposure, possibly due to the relatively brief (mean = 5 days, median = 3 days) period of exposure.

Repeated dose toxicity  
**Source:** German rapporteur  
**Flag:** Risk Assessment  
07-JUL-2005 (775)

**Remark:** ACUTE TOXICITY  
The acute toxicity to humans when benzene is inhaled has been described as being as follows:

20,000 ppm 5-10 minutes exposure can give fatalities  
7,5000 ppm 30 minutes exposure may be fatal  
1500 ppm 60 minutes exposure causes serious symptoms  
500 ppm 60 minutes exposure leads to symptoms of illness  
50-150 ppm 5 hours exposure causes headache, lassitude and weakness  
25 ppm 8 hours exposure causes no clinical effect.

Signs of toxicity following exposures are CNS depression, cardiac arrhythmia and respiratory failure resulting in death. At lower levels of exposure CNS effects are rapidly reversible.

The acute lethal dose of benzene to humans when ingested has been estimated to be about 10 mls (8.8g). The signs and symptoms of toxicity are typically those of narcotic solvent

type materials leading to profound CNS depression, collapse and death (WHO, 1993).  
Repeated dose toxicity  
**Source:** BP Chemicals Ltd LONDON  
**Flag:** Risk Assessment  
13-SEP-2000 (1252)

**Remark:** GENOTOXICITY IN HUMANS  
The finding of both structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells of those occupationally exposed to benzene has led to general acceptance that benzene can be a human clastogen (WHO, 1993). It is however acknowledged that methodological difficulties (e.g. adequate sample size and control groups, confounding by other factors especially age and smoking) make this a difficult area to study (Ashby & Richardson, 1985).

Chromosome aberrations have been reported in individuals who have previously suffered benzene induced haemopathy resulting from high benzene exposures (125-532 ppm for 1-22 years; Forni et al. 1971). Some studies have indicated genotoxic effects in individuals exposed to lower concentrations of benzene. Picciano (1979) has reported increases in chromosomal aberrations in workers exposed to concentrations <10 ppm and Sarto et al. (1984) reported an increased frequency of chromosome aberrations but not of sister chromatid exchanges (SCEs) in benzene production workers exposed to 0.2-12.4 ppm. Yardley-Jones et al. (1990) have reported a slight increase in lymphocyte chromosomal aberrations in oil refinery workers (48 workers and 29 controls) largely exposed to <1 ppm 8-hour TWA benzene. There was however no increase in SCEs (Yardley-Jones et al. 1988).

In contrast, other studies of workers exposed to benzene have shown no excess of chromosome aberrations (Watanabe et al. 1980; De Jong et al. 1988) or SCEs (Seiji et al. 1990; Watanabe et al. 1980). The finding of an excess of chromosomal aberrations in milk road tanker drivers (not specifically exposed to benzene) compared to petrol tanker crews (exposed to 6.5 ppm 8-hour TWA benzene) illustrates the difficulties of execution and interpretation of human cytogenetic studies in the occupational toxicology field (Berlin, 1985).

Used for Risk Assessment  
**Source:** BP Chemicals Ltd LONDON  
**Flag:** Risk Assessment  
21-AUG-2000 (47) (280) (365) (891) (998) (1028) (1239) (1252) (1292) (1293)

**Remark:** CARCINOGENIC EFFECTS

## Introduction

Populations exposed occupationally to benzene have been widely studied. Both case studies and epidemiological studies have been reported leading to the conclusion that benzene is a human leukaemogen (IARC, 1982; IARC, 1987; WHO, 1993). On the basis of such evidence benzene has been classified as a category 1 carcinogen within the EC and as a group 1 carcinogen ("The agent is carcinogenic to humans") by IARC.

The evidence is clear that at high concentrations (>100 ppm) exposure to benzene for a number of years can lead to the development of acute non-lymphocytic leukaemia (ANLL), predominantly the acute myeloid form.

The evidence that benzene might induce other haematopoietic and lymphatic malignancies is however very much weaker. Lymphopoietic malignancies have also been associated with benzene exposure but often under circumstances in which confounding factors have been involved or in which the evidence has been very much weaker than for ANLL. Associations have been reported between benzene exposure and both Hodgkins and non-Hodgkins lymphomas (Young, 1989; Vianna & Polan, 1979). However, a lack of an association between lymphoma and benzene work has also been shown (Smith & Lickiss, 1980). The cohort studies examining benzene exposure have not identified notably increased incidences of lymphoma.

Cases of benzene exposed workers developing multiple myeloma (Goldstein, 1990; Aksoy, 1980) have been reported but the reported excesses of multiple myeloma reported in the cohort studies of Rinsky (Rinsky et al. 1987) have not been confirmed in other cohort studies.

Investigation of workers heavily exposed to benzene when it was used as a solvent in the manufacture of shoes and other leather goods gave evidence that bone marrow supression due to benzene was sometimes followed by the development of leukaemia (Aksoy et al. 1972; Vigliani & Forni 1976; Aksoy 1978). Although acute non-lymphocytic leukaemia (ANLL) has been the predominant form reported there have been reports of acute lymphocytic leukaemia and chronic leukaemias (Aksoy, 1978, Aksoy et al. 1976).

Much of the basic evidence for the link between high benzene exposures and ANLL came from the large worker population in the Turkish leather working industry. Of an estimated 28,500 workers reported in the shoe, slipper and handbag industry in Istanbul, in which benzene was used as a solvent, 26 were admitted to hospital between 1967 and 1973 with acute leukaemia or preleukaemia. The working conditions in the industry were poor with small, unhygienic and badly ventilated workshops and the concentrations of benzene were reported to reach a maximum of 210-650 ppm when benzene containing glues were being used. Acute myeloblastic leukaemia was seen in 14 of these 26 patients who had been exposed for 1-15 years (mean of 9.7 years). The annual incidence of acute or preleukaemia was at least 13/100,000 which was statistically significantly greater ( $P > 0.02$ ) than the overall incidence of leukaemia in the general population (6/100,000) (Aksoy et al. 1974).

A number of cohort studies have been carried out on worker populations that have been exposed to benzene. These have however generally had limitations, many of which have been beyond the control of the investigators. Such limitations have been imposed for example by the difficulties in estimating historic exposures.

The principal studies are outlined in the following entries.

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (15) (19) (423) (552) (553) (940) (1058) (1214) (1252) (1302)

**Remark:** DOW CHEMICAL WORKERS

The mortality rates for 594 Michigan workers exposed to various compounds, including benzene, on or after January 1, 1940, were followed to the end of 1973 and compared with those for the US white male population adjusted for age, cause and calendar-year. The estimated benzene exposure concentrations ranged from 0.3-35.5 ppm.

No statistically significant increases were recorded for total death rate or any specific causes of death (Ott et al. 1978). This study was updated to the end of 1982. The original cohort of 594 workers exposed for at least 1 month between 1940 and 1970 was extended to include an additional 362 workers exposed up to 1978. The duration of exposure ranged from  $< 1$  to  $\geq 15$  years with a mean of 7 years. Those lost to follow-up (15) were withdrawn on the date they were last known to be alive. Data were analysed both with and without data from workers with possible confounding exposures to arsenic, asbestos and high levels of vinyl

chloride. Excluding those with these possible confounding exposures, death from all causes was not increased (O/E=200/242.3; SMR=83; 95% CI=72-95) but a significant excess of skin cancer death was recorded (O/E=4/0.9; SMR=441; 95% CI=121-1138). The excess of skin cancer however was suggested not to be related to benzene exposure on account of the cases all being in the lowest exposure category, with no common characteristic suggesting a link to exposure and no parallel findings in other cohort studies. No significant results were found for other specific causes of death.

Leukaemia was increased (O/E=3/1.9; SMR=162; 95% CI=33-461) but this was not statistically significant (Bond et al. 1986).

**Source:**  
13-DEC-1996

BP Chemicals Ltd LONDON

(133) (862)

**Remark:**

CMA CHEMICAL WORKERS

Wong (1987a,b) has reviewed the mortality experience of 3536 workers from seven plants in the USA, who were continuously exposed to benzene for at least six months between 1946 and 1976. Estimates of benzene exposure data were based, where possible, upon industrial hygiene data and workers were assigned to three exposure classes based on cumulative estimated 8-hour TWA values (<15 ppm years, 15-59.9 ppm years and >=60 ppm years).

Mortality for lymphatic and haematopoietic cancer combined showed five deaths in each exposure category and increasing SMRs with increasing estimates of cumulative dose (35, 91, 147 and 175 respectively for the control low, medium and high exposure groups; p=0.02).

Only six leukaemia deaths were observed and none of these was from acute myeloid leukaemia. The leukaemia SMRs were 0, 97, 78 and 275 for the control low, medium and high exposure groups respectively (p=0.02). The exposure response relationship, between cumulative benzene exposure and non-Hodgkin's lymphoma was of marginal statistical significance.

**Source:**  
13-DEC-1996

BP Chemicals Ltd LONDON

(1271) (1272)

**Remark:**

OTHER CHEMICAL WORKERS

In a retrospective cohort study of 259 men employed in a chemical plant for at least a year between 1947 and 1960, four deaths from lymphatic and haematopoietic cancer were observed. When analysed as combined lymphatic/haematopoietic cancer, this was statistically significant (SMR=377; 95% CI=109-1024). Three of these

were  
due to leukaemia (of which two were ANLL and one chronic lymphatic leukaemia) and one was due to multiple myeloma. SMRs were not given for leukaemia and multiple myeloma. No cases of aplastic anaemia were found and benzene exposures were not quantified. They were however likely to be high in  
some workers (Decoufle et al. 1983).

**Source:**  
13-DEC-1996

BP Chemicals Ltd LONDON

(286)

**Remark:**

PLIOFILM RUBBER WORKERS

The vital status of a cohort of 1165 white males occupationally exposed for at least 1 day between January 1, 1940 and December 31, 1965 in the production of a natural rubber cast film at two localities in Ohio, was followed to December 31, 1981. The expected numbers of cause-specific deaths within 5-year age and 5-year calendar periods were based on death rates for the US white male population specific for the same 5-year age and calendar periods. At follow-up, 16 members of the cohort (1.4%) were unaccounted for but were assumed to be alive on December 31, 1981.

No statistically significant increases in total mortality (O/E=330/331.6; SMR=99.5) or deaths from malignant neoplasms (O/E=69/66.8; SMR=103) were found. Deaths from all lymphatic and haemopoietic neoplasms were significantly in excess (O/E=15/6.6; SMR=227; 95% CI=127-376); this being due mainly to excess numbers of leukaemia deaths (O/E=9/2.7; SMR=337; 95% CI=154-641) and multiple myeloma (O/E=4/1; SMR=409; 95% CI=110-1047). The leukaemia deaths were of heterogenous histological types (1 chronic myelogenous, 2 unspecified myelogenous and 6 acute myelogenous [ANLL]).

Industrial hygiene records gave background information on the atmospheric concentrations of benzene after 1946 and, for the most part, employees' 8-hr time-weighted average exposures appeared to be within the limits of the standard in effect at any given time (10-100 ppm). Occasional increases above these levels were reported, however, workers were required to wear respirators when the recommended limit was exceeded. The efficiency of these respirators was not reported.

The cumulative exposure for each cohort member was estimated from historical air-sampling data or from interpolation on the basis of existing data. The cohort was then divided according to levels of cumulative exposure; a level of 400 ppm-years equating to a mean annual exposure of 10 ppm over

a 40-year working life. The SMRs for leukaemia deaths (SMRs=109, 322, 1186 and 6637) were found to markedly and progressively increase with a corresponding increase in cumulative exposure (1 ppm-day -39.99 ppm-years; 40-199.99 ppm-years; 200-399.99 ppm-years and >400 ppm-years respectively). These estimated cumulative exposure sub-groups contained 2, 2, 2 and 3 leukaemia deaths respectively. When halving or doubling these exposure ranges, the resultant SMRs continued to show a strongly positive trend of increasing risk with increasing exposure.

The time to death ranged from <5 to >30 years but no apparent pattern was evident.

A case-control analysis, conducted by comparing each cohort leukaemia death with ten controls matched for year of birth and first year of employment, indicated that the mean cumulative exposure was higher for the cases than the controls (254 and 50 ppm-years respectively), the average duration of exposure was longer (8.7 and 2.6 years respectively) and there was a difference in the rates of exposure (approximate averages of 24 and 16.5 ppm benzene/day respectively). It was suggested that the controls were selected from cohort members still alive at the time of death of the corresponding case.

The authors noted that, although hydrochloric acid, soda ash, natural rubber and small amounts of plasticizers were used, benzene was the only chemical in the plants that could reasonably be associated with haematologic toxicity (Rinsky et al. 1987). This study is an update of previous work (Infante et al. 1977; Rinsky et al. 1981).

Partly on the basis that it is a study in which benzene exposures appear not to have been confounded by other significant chemical exposures, the Rinsky et al. 1987 study has been widely cited. It has also been the basis of a number of quantitative risk assessment extrapolations which have attempted to project the leukaemia risk of benzene. However, this study has a number of limitations, largely concerning the estimation of worker exposure to benzene. No industrial hygiene data were available for the period 1940-1946 during which 6/9 leukaemia case workers were employed. It has been suggested that the Rinsky et al. 1987 estimates of exposure prior to 1946 were severe underestimates because of the following factors (Paustenbach et al. 1992; Crump & Allen, 1984; Cody et al. 1993; CEFIC 1988):-

An extensive exhaust ventilation system was installed at one plant in 1946. Exposures would have been expected to have

been higher without such a system than were subsequently shown by industrial hygiene monitoring and used as a basis for determining worker exposures.

Although mortality from aplastic anaemia was not reported in the study, blood dyscrasias including aplastic anaemia were not uncommon amongst the work force at the Pliofilm plants indicating that some high exposures had taken place.

Review of worker haematological data and comparison with individual "pre-employment" data suggest that workers in the 1940s experienced clinically detectable bone marrow depression during their initial period of work in benzene areas of the plant. This information supports the view that benzene exposures were higher during this period than those estimated by Rinsky et al. 1987.

Account had not been taken of the possible effect of short term peak exposures to benzene.

No account was taken of dermal exposure to benzene.

Tyre building work was carried out at one Pliofilm site with the possibility that benzene exposures had been higher there.

War production work during the period 1940-1945 is likely to have been associated with higher exposures and longer working hours.

It has also been suggested that the case control study may be compromised by poor matching of the exposure history of the control group compared to the cases (cases mean period exposed 8.9 years, controls mean period exposed 2.6 years) (CEFIC, 1988).

**Source:** BP Chemicals Ltd LONDON  
13-DEC-1996 (180) (221) (264) (558) (873) (938) (939) (1251)

**Remark:** CHINESE INDUSTRIAL WORKERS

A retrospective study of 28,460 workers selected from 233 painting, shoe-making, rubber synthesis, and adhesives and organic synthesis factories in 12 cities in China who had worked for at least 6 months between January 1, 1972 and December 31, 1981, was conducted. The control cohort of 28,257 individuals who had worked in the same cities for at least 6 months, was chosen from 83 machine production, textile and cloth factories in which there was no known exposure to benzene or other occupational carcinogens. Thirty cases of leukaemia (of whom 25 had died) were identified in the benzene cohort and four (all of whom had

died) in the control group, giving mortality rates from leukaemia of 14/100,000 person-years and 2.01/100,000 person-years respectively. The numbers of leukaemia deaths were significantly in excess for both male and female workers [SMR=501 (95% CI=292-802) and 830 (95% CI=358-1635) respectively]. The mean age of the leukaemia patients was lower in the exposed group and the average latency period was 11.4 years (ranging from 0.8-49.5 years). The benzene concentrations, as determined in a grab sampling programme, ranged from 7-1100 mg/m<sup>3</sup> (~3-340 ppm) but were mainly in the range of 50-500 mg/m<sup>3</sup> (~16-160 ppm). Chronic benzene poisoning and aplastic anaemia were identified in 196 exposed workers, seven of whom died from leukaemia. The leukaemia mortality rate among these poisoning cases was 700.7/100,000 person-years; 49 times greater than that in the benzene workers (Yin et al. 1987). It has been commented that little detail has been given of the methods used to take account of the possible confounding effects of smoking (WHO, 1993).

**Source:** BP Chemicals Ltd LONDON (1252) (1298)  
13-DEC-1996

**Remark:** Chromosomenuntersuchungen bei 10 gegenueber Benzol exponierten Beschaeftigten in der Benzol-Fabrikation zeigten 1-3 % Chromosomenaberrationen. Die Phenolausscheidung im Urin lag bei 31-65 mg/l.

**Source:** BASF AG Ludwigshafen (388)  
13-DEC-1996

**Remark:** Epidemiological studies have been conducted on workers in the tire industry (Mc. Mitchael et Al. 1975) and in the shoe factories (Aksoy et Al., 1974) where benzene is used extensively. Among workers who died from exposure to benzene, death was caused by either leukaemia or aplastic anemia, in aprox. equal proportions.

**Source:** REPSOL PETROLEO, S.A. MADRID (412)  
13-DEC-1996

**Remark:** A hematological investigation was carried out on 147 workers (employed for +1year) exposed to high benzene levels (320-470ppm). Abnormalities were noted in at least one parameter in 73%, the most common one being thrombocytopenia, which occurred in 62% followed by anemia 35% and leucopenia 32%.

**Source:** REPSOL PETROLEO, S.A. MADRID (994)  
13-DEC-1996

**Remark:**

## BONE MARROW DEPRESSION

Bone marrow depression results in a spectrum of haematological changes depending upon the degree of exposure and to individual susceptibility. Thrombocytopaenia, pancytopaenia, and aplastic anaemia may result.

Aksoy (1972) reported on 32 individuals who had been exposed to 150-650 ppm benzene for periods of 4 months-15 years and who had pancytopaenia with hypoplastic, hyperplastic or normoplastic marrow. Eight of the 32 were thrombocytopaenic with associated haemorrhage and infection. Severe or fatal aplastic anaemia seems to be associated with high exposure (>200 ppm) (Aksoy, 1972; Vigliani & Forni, 1976).

Much of the literature on haematological effects of benzene relates to exposures that took place in the 1930s and 1940s and consequently had poor characterization of benzene exposure (WHO, 1993). The haematotoxic effects of benzene appear to occur with exposures to >30 ppm (ECETOC, 1984). More recent studies show no (or marginal effects) after 8 hour TWA exposures of 2-30 ppm, <0.1-25 ppm, <1.0-10 ppm and 0.01-1.4 ppm (Townsend et al. 1978; Tsai et al. 1983; Yardley-Jones et al. 1988; Collins et al. 1991).

As benzene is toxic to bone marrow it would not be unexpected that it could have an effect on immune function.

Studies of immune function in workers have however usually been compromised by confounding exposures (WHO, 1993).

**Source:**

06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn  
(19) (224) (320) (1149) (1161) (1216) (1252) (1292)

**Remark:**

## GENOTOXICITY IN HUMANS

The finding of both structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells of those occupationally exposed to benzene has led to general acceptance that benzene can be a human clastogen (WHO, 1993). It is however acknowledged that methodological difficulties in elder (1965-1984) cytogenetic studies (e.g. adequate sample size and control groups, confounding by other factors especially age and smoking) make this a difficult area to study (Ashby & Richardson, 1985).

Chromosome aberrations have been reported in individuals who have previously suffered benzene induced haemopathy resulting from high benzene exposures (125-532 ppm for 1-22 years; Forni et al. 1971). Some studies have indicated genotoxic effects in individuals exposed to lower concentrations of benzene. Picciano (1979) has reported significant increases in chromosomal aberrations in 52

workers exposed to concentrations < 10 ppm (control n=44) and Sarto et al. (1984) reported an increased frequency of chromosome aberrations but not of sister chromatid exchanges (SCEs) in benzene production workers exposed to 0.2-12.4 ppm. Yardley-Jones et al. (1990) have reported a slight increase (significant using Fisher's exact test) in lymphocyte chromosomal aberrations in oil refinery workers (48 workers and 29 controls) largely exposed to <10 ppm 8-hour TWA benzene. There was however no increase in SCEs (Yardley-Jones et al. 1988).

The distribution of the breakpoints in the karyotypes of 56 workers occupationally exposed to benzene (<10 ppm, 6 h/d for 10-20 years) was investigated in cultured lymphocytes. A significant increase (control n=20) in chromosome aberrations was observed. The breakpoints in the karyotypes accumulated mainly on chromosome 2, 4, and 7 (Sasiadek, 1992).

In contrast, other studies of workers exposed to benzene have shown no excess of chromosome aberrations (Watanabe et al. 1980; De Jong et al. 1988) or SCEs (Seiji et al. 1990; Watanabe et al. 1980). The finding of an excess of chromosomal aberrations in milk road tanker drivers (not specifically exposed to benzene) compared to petrol tanker crews (exposed to 6.5 ppm 8-hour TWA benzene) illustrates the difficulties of execution and interpretation of human cytogenetic studies in the occupational toxicology field (Berlin, M., Am. J. Ind. Med. 7, 365-373 (1985)).

Used for Risk Assessment

**Source:** Deutsche Shell Chemie GmbH Eschborn  
21-AUG-2000 (47) (280) (365) (891) (998) (1003) (1028) (1239) (1292) (1293)

**Remark:** CARCINOGENIC EFFECTS

#### Introduction

Populations exposed occupationally to benzene have been widely studied. Both case studies and epidemiological studies have been reported leading to the conclusion that benzene is a human leukaemogen (IARC, 1982; IARC, 1987; WHO, 1993). On the basis of such evidence benzene has been classified as a category 1 carcinogen within the EC and as a group 1 carcinogen ("The agent is carcinogenic to humans") by IARC.

The evidence is clear that at high concentrations (>100 ppm) exposure to benzene for a number of years can lead to the development of acute non-lymphocytic leukaemia (ANLL), predominantly the acute myeloid form.

The evidence that benzene might induce other haematopoietic and lymphatic malignancies is however very much weaker. Lymphopoietic malignancies have also been associated with benzene exposure but often under circumstances in which confounding factors have been involved or in which the evidence has been very much weaker than for ANLL. Associations have been reported between benzene exposure and both Hodgkins and non-Hodgkins lymphomas (Young, 1989; Vianna & Polan, 1979). However, a lack of an association between lymphoma and benzene work has also been shown (Smith & Lickiss, 1980). The cohort studies examining benzene exposure have not identified notably increased incidences of lymphoma.

Cases of benzene exposed workers developing multiple myeloma (Goldstein, 1990; Aksoy, 1980) have been reported but the reported excesses of multiple myeloma reported in the cohort studies of Rinsky (Rinsky et al. 1987) have not been confirmed in other cohort studies.

Investigation of workers heavily exposed (150-650 ppm, 4 months-15 years) to benzene when it was used as a solvent in the manufacture of shoes and other leather goods gave evidence that bone marrow suppression due to benzene was sometimes followed by the development of leukaemia (Aksoy et al. 1972; Vigliani & Forni 1976; Aksoy 1978). Although acute non-lymphocytic leukaemia (ANLL) has been the predominant form reported there have been reports of acute lymphocytic leukaemia and chronic myeloid leukaemias (Aksoy, 1978, Aksoy et al. 1976).

Much of the basic evidence for the link between high benzene exposures and ANLL came from the large worker population in the Turkish leather working industry. Of an estimated 28,500 workers reported in the shoe, slipper and handbag industry in Istanbul, in which benzene was used as a solvent, 26 were admitted to hospital between 1967 and 1973 with acute leukaemia or preleukaemia. The working conditions in the industry were poor with small, unhygienic and badly ventilated workshops and the concentrations of benzene were reported to reach a maximum of 210-650 ppm when benzene containing glues were being used. Acute myeloblastic leukaemia was seen in 14 of these 26 patients who had been exposed for 1-15 years (mean of 9.7 years). The annual incidence of acute or preleukaemia was at least 13/100,000 which was statistically significantly greater ( $P>0.02$ ) than the overall incidence of leukaemia in the

general population (6/100,000) (Aksoy et al. 1974).

A number of cohort studies have been carried out on worker populations that have been exposed to benzene. These have however generally had limitations, many of which have been beyond the control of the investigators. Such limitations have been imposed for example by the difficulties in estimating historic exposures.

The principal studies are outlined in the following entries.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (15) (19) (423) (552) (553) (940) (1058) (1214) (1252) (1302)

**Remark:** DOW CHEMICAL WORKERS

The mortality rates for 594 Michigan workers exposed to various compounds, including benzene, on or after January 1, 1940, were followed to the end of 1973 and compared with those for the US white male population adjusted for age, cause and calendar-year. The estimated TWA benzene exposure concentrations ranged from 0.3-35.5 ppm.

No statistically significant increases were recorded for total death rate or any specific causes of death (Ott et al. 1978). This study was updated to the end of 1982. The original cohort of 594 workers exposed for at least 1 month between 1940 and 1970 was extended to include an additional 362 workers exposed up to 1978. The duration of exposure ranged from <1 to >= 15 years with a mean of 7 years. Those lost to follow-up (15) were withdrawn on the date they were last known to be alive. Data were analysed both with and without data from workers with possible confounding exposures to arsenic, asbestos and high levels of vinyl chloride. Excluding those with these possible confounding exposures, death from all causes was not increased (O/E=200/242.3; SMR=83; 95% CI=72-95) but a significant excess of skin cancer death was recorded (O/E=4/0.9; SMR=441; 95% CI=121-1138). The excess of skin cancer however was suggested not to be related to benzene exposure on account of the cases all being in the lowest exposure category, with no common characteristic suggesting a link to exposure and no parallel findings in other cohort studies. No significant results were found for other specific causes of death.

In the cohort less exclusions (exposure to arsenic, asbestos, or high levels of vinyl chloride) leukaemia was increased (O/E=3/1.9; SMR=162; 95% CI=33-461) but this was not statistically significant (Bond et al. 1986).

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(133) (862)

**Remark:** CMA CHEMICAL WORKERS

Wong (1987a,b) has reviewed the mortality experience of 3536 workers from seven plants in the USA, who were continuously exposed to benzene for at least six months between 1946 and 1976. Estimates of benzene exposure data were based, where possible, upon industrial hygiene data and workers were assigned to three exposure classes based on cumulative estimated 8-hour TWA values (<15 ppm years, 15-59.9 ppm years and >=60 ppm years).

Mortality for lymphatic and haematopoietic cancer combined showed five deaths in each exposure category and increasing SMRs with increasing estimates of cumulative dose (35, 91, 147 and 175 respectively for the control low, medium and high exposure groups; p=0.02).

Only six leukaemia deaths were observed and none of these was from acute myeloid leukaemia. The leukaemia SMRs were 0, 97, 78 and 275 for the control low, medium and high exposure groups respectively (p=0.02). The exposure response relationship, between cumulative benzene exposure and non-Hodgkin's lymphoma was of marginal statistical significance.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1271) (1272)

**Remark:** OTHER CHEMICAL WORKERS

In a retrospective cohort study of 259 men employed in a chemical plant for at least a year between 1947 and 1960, four deaths from lymphatic and haematopoietic cancer were observed. When analysed as combined lymphatic/haematopoietic cancer, this was statistically significant (SMR=377; 95% CI=109-1024). Three of these were due to leukaemia (of which two were ANLL and one chronic lymphatic leukaemia) and one was due to multiple myeloma. SMRs were not given for leukaemia and multiple myeloma. No cases of aplastic anaemia were found and benzene exposures were not quantified. They were however likely to be high in some workers (Decoufle et al. 1983).

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(286)

**Remark:** CHINESE INDUSTRIAL WORKERS

A retrospective study of 28,460 workers selected from 233 painting, shoe-making, rubber synthesis, and adhesives and organic synthesis factories in 12 cities in China who had

worked for at least 6 months between January 1, 1972 and December 31, 1981, was conducted. The control cohort of 28,257 individuals who had worked in the same cities for at least 6 months, was chosen from 83 machine production, textile and cloth factories in which there was no known exposure to benzene or other occupational carcinogens. Thirty cases of leukaemia (of whom 25 had died) were identified in the benzene cohort and four (all of whom had died) in the control group, giving mortality rates from leukaemia of 14/100,000 person-years and 2.01/100,000 person-years respectively. The numbers of leukaemia deaths were significantly in excess for both male and female workers [SMR=501 (95% CI=292-802) and 830 (95% CI=358-1635) respectively]. The mean age of the leukaemia patients was lower in the exposed group and the average latency period was 11.4 years (ranging from 0.8-49.5 years). The benzene concentrations, as determined in a grab sampling programme, ranged from 7-1100 mg/m<sup>3</sup> (3-340 ppm) but were mainly in the range of 50-500 mg/m<sup>3</sup> (16-160 ppm). Chronic benzene poisoning and aplastic anaemia were identified in 196 exposed workers, seven of whom died from leukaemia. The leukaemia mortality rate among these poisoning cases was 700.7/100,000 person-years; 49 times greater than that in the benzene workers (Yin et al. 1987). It has been commented that little detail has been given of the methods used to take account of the possible confounding effects of smoking (WHO, 1993).

**Source:** Deutsche Shell Chemie GmbH Eschborn (1252) (1298)  
06-JAN-1997

**Remark:** COKE OVEN WORKERS

A number of cohort studies have looked at mortality in coke oven workers. No excesses of leukaemias and lymphomas combined or of leukaemias respectively were found in North American or Dutch coke oven workers (Redmond et al. 1972; Swaen et al. 1991). Exposures to benzene were not reported in these studies.

Preliminary results from a total 6520 British male coke plant workers have been reported (Hurley et al. 1991). Personal air sampling from two cohorts of benzole workers (84 and 307 workers respectively) showed benzene concentrations of <0.19-15 ppm. Contemporary mean 8 hour time weighted averages for similar workers in the UK during the 1980s were 1.3 ppm. No increased risk of mortality from leukaemia has been observed in either cohort (SMR=98, 95% CI=2-429, 1 death and SMR=76, 95% CI=1-192, 1 death). For two cohorts of coke oven workers (total 3349 workers) leukaemia mortality had SMRs of 34, 95% CI=0-186, 1 death and 35 95% CI=1-192, 1 death respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Flag:** Risk Assessment (541) (931) (1103)  
06-JAN-1997

**Remark:** Odor threshold = 1.5 ppm  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (401)

**Remark:** This is a review of hematotoxic and carcinogenic properties of benzene, supplemented with a summary of case or series findings from the author's patients. The suggested effects of benzene exposure and topics discussed include: aplastic anemia or pancytopenia; benzene - hepatitis - aplastic anemia syndrome; leukemia; leukemia in pancytopenia patients with chronic benzene toxicity; genetic relationships and individual susceptibility in leukemia; other cancers - malignant lymphomas, multiple myeloma, lung cancer. The conclusions of the author are suggestive rather than definitive.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (16)

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (304)

**Remark:** Repeated dose toxicity  
The authors describe clinical findings of ten Dow Chemical Company employees exposed for several years to over 25 ppm, 8-hour TWA benzene. The initial high exposure of these workers had started more than 16 years previous to this report. Some alterations in hemoglobin and mean corpuscular volume were observed, but no other significant blood or bonemarrow abnormalities. Since the time of high exposures in 1963, the clinical findings have generally tended to return to normal values. All ten employees were included in the mortality and morbidity studies conducted at

Dow and published as separate reports.  
**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (361)

**Remark:** Repeated dose toxicity  
This is a cross-sectional study of 332 workers in three fast-press rotogravure printing plants in NYC in 1938. Benzene concentrations ranged from 11 to 1060 ppm in the three plants. There were 48 area samples analyzed and were probably 8-hour TWAs, although methods are not specified in this report. There was also dermal exposure as the workers used benzene to wash the ink off their hands and arms. Physical examination and complete blood studies were completed on 102 benzene workers and partial studies on the remainder; there were 81 nonbenzene exposed workers available for some comparisons. There were 130/332 cases

with variable degrees of benzene poisoning. The findings from the blood studies are described.

The study shows that high benzene [usually > 320 mg/m<sup>3</sup> (100 ppm) as TWA for months to years] may lead to marked decrease in white blood cell count.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (443)

**Remark:** Repeated dose toxicity  
This is an analysis of white and red cell counts as well as hemoglobin of 459 benzene-exposed workers between 1940 and 1975. These workers are a subset of about 7200 rubber workers from the Pliofilm cohort at St. Mary's in Akron, Ohio. Positive correlations between fluctuations in blood count and benzene exposure were reported between 1940-1948 (mean estimated exposure = 75 ppm) but not from 1948-1975 (estimated exposure = 15-20 ppm). The authors suggest examination of the blood is unlikely to detect abnormalities at lower benzene exposures.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (621)

**Remark:** Repeated dose toxicity  
This report is a literature review of benzene. Topics discussed include animal models for benzene-induced leukemia, mechanism of benzene-induced toxicity and the role of benzene metabolites, studies in mutagenicity and teratogenicity, toxicity and carcinogenicity in humans, population exposures to benzene (especially exposure through water), and risk assessment. The review is particularly useful for historical purposes as the older literature is included. There are 13 pages of references, two of which go back to 1880.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (745)

**Remark:** Ten workers were exposed to a benzene spill that resulted in substantial excretion of phenol. Three months later, genotoxic abnormalities in peripheral lymphocytes of benzene-exposed workers and 11 controls were compared. Lymphocytes were examined for chromosomal damage (e.g., polyploidy, gaps, chromatid breaks, acentric fragments) and the frequency of sister chromatid exchange. Controls had slightly more chromosomal damage, but all values were in the normal range. Benzene-exposed workers had slightly more SCE with a slight exposure-response trend based on maximum

phenol excretion shortly after the spill. The authors concluded there was no evidence of any lasting chromosomal damage resulting from the acute benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn (213)  
06-JAN-1997

**Remark:** Peripheral blood lymphocytes of 34 workers exposed to benzene and toluene at a rotogravure plant and 34 controls matched by sex and age were analyzed from chromosomal aberrations. All chromosomal analyses were carried out blindly by the authors, and persons with viral diseases and recent vaccinations were excluded. Benzene concentrations ranged from 131 ppm to 532 ppm. Among 10 workers exposed to benzene before 1952 and then to toluene, the proportion of

unstable and stable chromosomal aberrations were statistically significantly higher compared with workers exposed only to toluene. The frequencies of chromosomal aberrations were not statistically significantly different among workers exposed only to toluene and controls.

**Source:** Deutsche Shell Chemie GmbH Eschborn; German rapporteur  
**Flag:** Risk Assessment (363)  
13-OCT-2000

**Remark:** Chromosomal aberrations in cultured lymphocytes were studied in six groups of 65 workers exposed to benzene in motor fuels and a younger group of 15 patient controls. A moderate but statistically significant increase in frequency of aberrations was found in road tank drivers and workers at industrial gasworks, but not in ship tanker crews and employees at petrol filling stations. The range of TWA concentrations of benzene in each group were as follows: 0.02-0.31 ppm for petrol station employees; 0.01-4.13 ppm for road tank drivers delivering petrol; 0.33-23 ppm for crew members loading ship tankers (but frequency of exposure was less than road tanker drivers); 5-10 ppm for gasworks workers, but there were only occasional short-term exposures. No estimates were available for road tanker drivers delivering milk or for controls. The authors consider it unlikely that benzene is the main reason for aberrations in the road tanker drivers since there was no difference between those delivering petrol and those delivering milk.

**Source:** Deutsche Shell Chemie GmbH Eschborn (371)  
06-JAN-1997

**Remark:** The white blood cell count and frequency and size of lymphocyte micronuclei of 15 gasoline pump mechanics were compared to 15 construction worker controls. The male subjects in the two groups were similar in age and smoking. Pump mechanics were exposed to 1 mg/m<sup>3</sup> benzene (8-hour TWA, 10-minute peaks as high as 20 mg/m<sup>3</sup>). The leukocyte count was higher in the exposed group. Beta-cell micronuclei were larger and more frequent compared to controls, but

there  
was no apparent occupational effect on T- cell lymphocyte  
micronuclei. Most human lymphocytic tumors originate from  
Beta-lymphocytes and appear more sensitive to mutagens than  
T-cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn (520)  
06-JAN-1997

**Remark:** Lymphocytes from peripheral blood of 52 benzene exposed  
workers and 44 controls were scored for cytogenetic  
changes. Exposed and controls were matched on sex and time  
period of analysis, but were not matched on age or smoking.

There were no apparent differences in the percentage of  
chromatid breaks and total abnormal cells between the two  
groups. However, the benzene exposed group had twice the  
percentage of chromosome breaks, three times the percentage  
of marker chromosomes, and 10 times the percentage of both  
breaks and marker chromosomes. Data collected between 1945  
and 1978 suggest 8-TWA concentrations were about 2-3 ppm  
benzene (25 ppm for 15 minutes and 50 ppm for 2-3 minutes  
samples during specific operations, and possibly > 100  
ppm.)

Ethylbenzene concentrations were about 2-10 ppm TWA, 25 ppm  
ceiling, and > 100 ppm excursions.

**Source:** Deutsche Shell Chemie GmbH Eschborn (617)  
06-JAN-1997

**Remark:** Chromosomes from peripheral blood leukocytes of three  
groups of factory workers exposed to benzene were examined.  
Results were compared with on-site as well as population  
controls without benzene exposure. The exposed workers in  
Group 1 had a higher proportion of stable chromosomal  
abnormalities than controls, but there were no apparent  
differences in Groups 2 and 3. Population controls had  
fewer stable chromosomal abnormalities than both exposed  
and controls in Group 2. There was no apparent differences  
in unstable chromosomal abnormalities between exposed and  
controls in any of the groups. Rings and dicentrics were  
lower in the population controls than in workers, were  
highest in Group 2 exposed and controls, and higher in  
Group 1 exposed than controls. Actual exposures to benzene  
were not known, but were probably less in Group 3. The  
reasons for the observed differences could be due to  
environmental factors, but differences in age, small  
numbers  
of counted cells, and perhaps inadequate comparison groups  
might explain some of these results.

**Source:** Deutsche Shell Chemie GmbH Eschborn (1147)  
06-JAN-1997

**Remark:** Results of cytogenetic analyses of peripheral blood lymphocytes from 58 benzene exposed shoe workers and 20 population controls without exposure to mutagenic or carcinogenic agents were compared. The exposed group had a higher percentage of smokers (68% vs. 35%) and alcohol drinkers (39% vs. 5%). The exposed group had higher frequencies of aberrations (breaks, gaps, rearrangements). Working period, smoking, and alcohol intake were thought to be unrelated to chromosomal changes in these groups (except for alcohol intake which was associated with increased frequency of breaks). No benzene exposure measurements were taken, but in Turkey the MAC is 20 ppm, and Aksoy indicated that maximum benzene exposure for Turkish shoemakers was between 210-610 ppm.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1169)

**Remark:** The authors calculated the incidence of leukemia among 28,500 shoeworkers chronically exposed to benzene in Istanbul. The maximum benzene concentrations to which these workers were exposed was 210-650 ppm. During the time period from 1967 to September 1973, 26 cases of acute leukemia or preleukemia were admitted to several clinics. Among these patients the duration of benzene exposure ranged from 1-15 years. The incidence of leukemia was calculated to be 13 per 100,000 workers, which is statistically significantly greater compared with the general population. The authors conclude that these data provide evidence for a leukemogenic effect of benzene in man.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (17)

**Remark:** Two cases of acute leukemia (lymphoblastic and myeloblastic) are presented. The commonality of these cases is that the two are related (one is the paternal uncle of the other) and both had chronic exposure to benzene from working as shoemakers. The thesis of this report is that genetic predisposition may be significant in susceptibility to benzene poisoning, and that some genetic factors were triggered by the 4-6 years of benzene exposure experienced at work by these patients.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (20)

**Remark:** This is a review of benzene as a carcinogen and covers some of the same issues covered in Aksoy (1989). Topics include: incidence of leukemia among Turkish shoemakers; types of leukemia (distribution of types of leukemia -- AML most common; leukemia preceded by pancytopenia; duration of exposure -- no clear relationship between duration of benzene exposure and type of leukemia; genetics and

individual susceptibility; safe workplace exposure limits for benzene -- ~1 ppm); other malignancies due to benzene --malignant lymphoma, multiple myeloma, lung cancer.

**Source:** Deutsche Shell Chemie GmbH Eschborn (14)  
06-JAN-1997

**Remark:** This is a review of hematotoxic and carcinogenic properties of benzene, supplemented with a summary of case or series findings from the author's patients. The suggested effects of benzene exposure and topics discussed include: aplastic anemia or pancytopenia; benzene - hepatitis - aplastic anemia syndrome; leukemia; leukemia in pancytopenia patientswith chronic benzene toxicity; genetic relationships and individual susceptibility in leukemia; other cancers -- malignant lymphomas, multiple myeloma, lung cancer. The conclusions of the author are suggestive rather than definitive.

**Source:** Deutsche Shell Chemie GmbH Eschborn (16)  
06-JAN-1997

**Remark:** This is a nested case-control study of lymphocytic leukemia.The 15 cases and 30 matched controls were identified from a cohort of rubber workers. Potential for exposure to benzenewas based on occupational titles and types of solvents used from records of product specifications and standard operating procedures. Cases were 4.5 times more likely thancontrols to have had direct exposure to benzene and other solvents. Cases were 6.7 times more likely than controls tohave been exposed to coal-based solvents, and 1.5 times morelikely than controls to have been exposed to petroleum-basedsolvents. Coal-based xylene appeared more potent than coal-based benzene with odds ratios of 5.5 and 3.0, respectively.

**Source:** Deutsche Shell Chemie GmbH Eschborn (45)  
06-JAN-1997

**Remark:** Mortality among more than 15,000 male employees of the Australian petroleum industry was examined from 1981 to 1989. All employees had at least 5 years of employment in the industry. Standardized mortality ratio (SMR) analysis indicated total cancer mortality was slightly lower comparedwith the national population. However, mortality due to lymphohematopoietic cancers, primarily leukemia (SMR=1.6; 95% confidence interval [CI] 0.6-3.4), was elevated, but this finding was not statistically significant. Mortality due to cancers of the pleura was also elevated (SMR=3.9; 95%CI=0.8-11.0), although two of the three cases were likely exposed to asbestos during employment in other industries.

**Source:** Deutsche Shell Chemie GmbH Eschborn (203)  
06-JAN-1997

**Remark:** This nested case-control study examined lymphohematopoietic cancer (LHC) mortality risk and longer term, lower benzene exposures among male marketing/distribution, marine and pipeline workers. Among the 31 cases studied, 16 had leukemia, 7 multiple myeloma and 8 non-Hodgkin's lymphoma. For each case, four male controls were selected from records from the same cohort, frequency matched to cases by decade of birth. Industrial hygienists estimated workplace exposures for benzene and total hydrocarbons for every job/location/era combination. For leukemia, family history of cancer and cigarette smoking were the two strongest risk factors, with cumulative benzene exposure showing no additional effect when considered in the same models. No exposure-response relationships were seen when benzene was categorized by several exposure metrics. The numbers of multiple myeloma and non-Hodgins lymphoma cases were too small to reliably characterize risk with logistic modelling.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (1017)

**Remark:** This is a review of benzene leukemias and a compilation of cases of leukemia and anemia associated with benzene exposure. The number of cases is from the published literature, unpublished reports from Northern Italy, and the authors experience in Milan. A synopsis of five new cases of benzene seen at the Institute of Occupational Health of Milan from 1964-1974 is provided.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
13-SEP-2000 (1215)

**Remark:** A quantitative exposure-estimating algorithm for benzene and total hydrocarbons was developed for a case control study of petroleum marketing and distribution workers. The algorithm used a multiplicative model to adjust recently measured quantitative exposure data to past scenarios for which representative exposure measurement data did not exist. This was accomplished through the development of exposure modifiers to account for differences in the workplace, the materials handled, the environmental conditions, and the tasks performed. Values for exposure modifiers were obtained empirically and through physical/chemical relationships. Dates for changes that altered exposure potential were obtained from archive records, retired employee interviews, and from current operations personnel. Exposure modifiers were used multiplicatively, adjusting available measured data to represent the relevant exposure scenario and time period. Changes in exposure modifiers translated to step changes in exposure estimates. Though limited by availability of data, a validation exercise suggested that the algorithm provided accurate exposure estimates for benzene (compared with measured data in industrial hygiene survey reports); the

estimates generally differed by an average of less than 20% from the measured values. This approach is proposed to quantify exposures retrospectively where there are sufficient data to develop reliable current era estimates and where a historical accounting of key exposure modifiers can be developed, but where there are insufficient historic exposure measurements to directly assess historic exposures.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(44)

**Remark:**

This paper describes cases of leukemia attributed to benzene exposure in Italy. Many of the cases of chronic benzene poisoning were due to use of glues containing benzene in the manufacture of shoes. Clinical details on 6 leukemia cases among 47 patients with benzene hemopathy are presented. All six cases were hemotologically similar, and the authors report that no cases of chronic myeloid or lymphatic leukemia have ever been observed in workers poisoned by benzene. Little data on benzene exposure concentrations are provided, although the authors report that

two of the six leukemia cases were exposed to levels ten times greater than the maximum allowable concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997

(1217)

**Remark:**

In a study conducted in the Love Canal area (New York, USA) by Heath (1983), the outcome of pregnancy was evaluated in populations living in the proximity of waste sites where within a total of at least 248 chemicals also benzene had been identified. No clear increase in occurrence of spontaneous abortion, birth defects, or low infant birth weight was observed in women living next to the canal. The study limitations of inadequate sample size and lack of exposure history preclude an assessment of significance of these findings.

This paper reviews study design issues and limitations associated with epidemiologic studies of health risks related to toxic waste exposure. Since the settings in which such studies must be performed are extremely diverse, epidemiologic approaches must be versatile. For any particular study, three fundamental requirements are to assess what toxic materials are present, understand how human exposure may occur, and objectively measure possible biologic effects. In assessing links between exposure and disease, epidemiologists must be particularly aware of: expected disease frequencies in relation to the size of populations studied, implications of long or varied disease latencies for study design and competing causes of disease and associated confounding variables. These concepts are illustrated by discussion of epidemiologic studies related to the Love Canal toxic waste dump site in Niagara Falls, NY.

**Source:** Deutsche Shell Chemie GmbH Eschborn; German rapporteur  
**Flag:** Risk Assessment  
25-OCT-2000 (496)

**Remark:** Hematological findings among 70 male employees of a coke oven by-product recovery facility with TWA benzene exposures of 33.6 mg/m<sup>3</sup> (10.5 ppm) and 21 unexposed males from the same facility were studied. A Cumulative exposure index was calculated and categorized as < 64 mg/m<sup>3</sup>-years (<20 ppm-years), 64-640 mg/m<sup>3</sup>-years (20-200 ppm-years), and > 640mg/m<sup>3</sup>-years (>200 ppm-years). WBC, RBC, and Hb values in exposed workers are similar to unexposed controls. A slight non-statistically significant trend towards lower Hb values was evident. The authors concluded that levels of about 32 mg/m<sup>3</sup> (10 ppm) had no effect on the haematologic parameters studied. This design averaged both exposures and time-sensitive blood counts; thus, transient effects of higher exposures [which reached an average of 534 mg/m<sup>3</sup> (167 ppm)] may have gone undetected.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (470)

**Remark:** Cytogenetic analyses were carried out in peripheral lymphocytes from 20 male workers exposed only to toluene in a rotogravure plant for more than 16 years. As compared with a group of 24 unexposed controls, significantly higher yields of chromatid breaks, chromatid exchanges and gaps were observed. The number of SCEs was significantly increased in smoking and non-smoking toluene-exposed workers compared with the corresponding control groups.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (82)

**Remark:** The authors studied cytogenetic parameters among 65 petrol service station attendants, petrol truck drivers, and marine (tanker) personnel. The results showed a significant excess of marker chromosomes (but no excess of chromosome breaks) in petroleum truck drivers when compared to lesser exposed petrol station staff. No significant excess was found in marine workers. The marine workers were exposed to higher mean and peak concentrations of benzene [21 and 74 mg/m<sup>3</sup> (6.6 and 23 ppm), respectively] when compared to the petroleum truck drivers [1.3 and 13 mg/m<sup>3</sup> (0.40 and 4.1 ppm), respectively]. However, the truck drivers were exposed more frequently. Thus, the meaning of the elevation in chromosome breaks was uncertain. The study was

blinded and 100 cells were scored per individual. There was no control for confounders such as age, smoking, etc. To clarify these issues, a followup analysis was performed which compared the petrol truck drivers to milk truck drivers. Thus, occupation was controlled for, and control for age, smoking, and other exposures was also performed. A third group of gas plant workers exposed to higher benzene levels [16-32 mg/m<sup>3</sup> (5-10 ppm)] was also studied. This followup analysis showed similar rates of chromosome breaks and marker chromosomes in the two groups of drivers. The more highly exposed gas plant workers also showed similar rates of chromosome breaks. In a subsequent presentation of these data, Berlin (1985) stated that all three groups (including the milk truck drivers) showed rates above historical population controls, but did not deviate from each other. Thus, benzene exposure was not related to the CAbr.

**Source:**  
06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(373)

**Remark:**

Cultured lymphocytes from 73 workers in chemical laboratories and the printing industry were found to have a significantly increased frequency of chromatid and isochromatid breaks, in comparison with 49 control subjects (42 adults and 7 children). An increase of the same magnitude was also found in 14 children, aged 4 days - 11 yr, of 11 women laboratory workers who had worked during pregnancy. The concentration of benzene and other chemicals to which these individuals were exposed was not reported. A significant correlation between age and frequency of chromosome aberrations was noted for both the exposed and control children but not for the adults. The frequency of sister-chromatid exchange was significantly increased in 12 technicians working in laboratories performing hormone analysis. 4 children of 2 female technicians working during pregnancy also had a significantly increased frequency of sister-chromatid exchange. The cause and biological significance of these findings are not yet known. In this study increased frequencies of chromosome breaks and of sister chromatid exchange were found in lymphocytes from 14 children of female workers exposed by inhalation to benzene and other organic solvents (doses not specified) during the pregnancy. No mention was made for the reasons of this investigation nor of whether mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

**Source:**  
**Flag:**  
25-OCT-2000

Deutsche Shell Chemie GmbH Eschborn; German rapporteur  
Risk Assessment

(382)

**Remark:** A cytogenetic study was performed on 13 patients (10 pancytopenia, 2 acute leukemia, and 1 praeleukemia) to examine the action of benzene on mitosis in haemopoiesis cells. All patients worked in shops using an adhesive containing benzene. Benzene concentrations ranged from 480-672 mg/m<sup>3</sup> (150-210 ppm). Various chromosomal abnormalities were observed among these workers and included: breakage, chromatid separation, chromatid interchanges, acromatic lesions, secondary constriction, dicentric chromosomes and acentric fragments. Other anomalies and signs of benzene toxicity were also observed.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
06-JAN-1997 (337)

**Remark:** This letter-to-the-editor describes a study which uses previously collected data from a case-control study of occupation and infertility to examine occupational solvent exposures and birth weight and length. A total of 2620 couples were eligible for the study. Data on birth weight and length were obtained from hospitals, and information on soilodemographic, occupational and medical history were obtained by survey (86% response rate). The results suggested average birth weight and length was not significantly less among solvent-exposed subjects compared with unexposed subjects. Results were adjusted for the effects of maternal age, smoking and drinking habits, time needed to conceive, and exposure of the spouse. The authors conclude the results do not support the hypothesis that exposure to organic solvents influences birth weight or length. Conclusions about benzene, however, are limited due to concomitant exposure to other chemicals, small sample size, and lack of quantification of exposure levels.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Flag:** Risk Assessment  
25-OCT-2000 (858)

**Remark:** Birth weight, prematurity, gestational age, and birth defects were assessed in 239 children exposed during gestational life to the Love Canal neighborhood and in 707 control children. The Love Canal site had been contaminated with at least 248 different chemicals. The population living in Love Canal was composed of two groups; those referred to as homeowners who lived in single family homes and were predominantly white, and those referred to as renters who lived in a low income apartment complex and were predominantly black. These two groups were matched with comparable groups in the same city and a questionnaire was administered by trained interviewers at a neighborhood site or in the home. Quantitative data on exposure were not available for study subjects. Mothers of exposed and control children were similar in socioeconomic status,

smoking, alcohol consumption and medication use during pregnancy. There was no significant difference in prematurity, but the prevalence of low birth weight babies (<2500 g) was increased in exposed compared to control children, and multiple regression analysis showed that for the homeowner group the adjusted odds ratio was 3.0 (95% confidence interval 1.3-7.0). Both exposed and control renter groups had a high prevalence of low birth weight babies and there was no difference between the two groups. Birth defects were increased in exposed homeowner and renter groups compared to control groups with adjusted odds ratios of 1.95 (1.03-3.72) and 2.87 (1.15-7.18), respectively. Conclusions about the role of benzene exposures in these findings are not possible due to concomitant exposure to numerous other chemicals and lack of

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Flag:** Risk Assessment

25-OCT-2000

(421)

**Remark:**

The Drake Superfund site in Clinton County, Pennsylvania, has been contaminated with the carcinogens B-naphthylamine, benzidine, and benzene. However, no quantitative data on exposure levels for these chemicals were available. The authors reviewed county-wide, age-adjusted, sex-, race-, and site-specific cancer mortality rates for the years 1950-1959, 1960-1969, and 1970-1979, and type-specific birth defects incidence rates for 1973-1978. During the 1970's a significantly increased number of bladder cancer deaths occurred among white males in Clinton County, and a significantly increased number of other cancer deaths occurred in the general population of Clinton and three surrounding counties. There were no statistically significant clusters of any specific birth defect or of all birth defects. Conclusions about the role of benzene exposures in this study are not possible due to concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels.

**Source:** Deutsche Shell Chemie GmbH Eschborn; German rapporteur

**Flag:** Risk Assessment

25-OCT-2000

(167)

**Remark:**

This article reviews the most frequently recurring chromosomal abnormalities associated with specific subgroups of acute nonlymphocytic leukemia. Current knowledge of specific genes that, due to chromosomal rearrangement, may be involved in the process of malignant transformation are also discussed. The authors make several conclusions which include: heritable fragile sites occur anywhere from 1 of 100 (for 16q22) to 1 of 700; common

fragile sites are ubiquitous; proto-oncogenes and other growth promoting genes are important in the malignant transformation process; and the FAB classification system can be refined based on cytogenetic findings. Literature on the frequency of deletions or loss of chromosome 5 and 7 in studies of patients who develop myelodysplastic syndromes or acute nonlymphocytic leukemia after antineoplastic therapy is approximately 90%.

**Source:**  
06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(108)

**Remark:**

The natural variability of chromosomal aberrations in control populations is an important factor to consider in interpreting studies of benzene exposure and chromosomal aberrations. The authors previously reported on a cytogenetic-epidemiological study of chromosomal aberration and sister-chromatid exchange (SCE) frequencies in peripheral blood lymphocytes (PBL) of a cohort of 353 healthy employees of the Brookhaven National Laboratory who were presumably unexposed to benzene or other chemicals. This sample has now been increased by adding living retirees from the laboratory in order to extend the age range represented and, incidentally, the representation of non-white subjects. In total, the data now include chromosomal aberration information from 108,950 cells and SCE information from 25,397 cells from 613 samples from 493 subjects.

Neither the mean frequencies of any of the chromosomal aberration types nor the mean frequency of SCE have changed notably through the addition of the new subjects and samples. The mean age at sampling of the population is now 43.1 years with a range of from 1.1 to 83.7 years. The results show no significant relationship of the frequency of any conventional aberration category to age with the single exception of the dicentric chromosome, which now shows a positive regression ( $p = 0.001$ ). The frequency of decentrics was 0.16 (aberration means per 100 cells). The raw mean frequency of SCE increased with age, although not after smoking was taken into account. The frequency of chromatid-type deletions was 0.81 (aberration means per 100 cells).

**Source:**  
06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(92)

**Remark:**

Repeated dose toxicity  
This report describes preliminary results of an unpublished study of the influence of low level benzene exposures on the blood cells. (Van Damme et al., 1991). The study examined all employees at a petrochemical plant who were working at the end of 1988 and had at least 5 haematological

examinations during 1967-1988. There were 484 employees with a total of 17,404 blood samples. Based on exposure data from 1978-1988, none of the workers were exposed to benzene levels higher than 6.4 mg/m<sup>3</sup> (2 ppm) since 1971. The results suggest an increased prevalence of leucopenia (defined as < 4000 cells/uL) for exposures of 0.64 to 1.92 mg/m<sup>3</sup> (0.2 to 0.6 ppm) which is well below no-effect level estimates of 64 mg/m<sup>3</sup> (20 ppm) for WBC effects reported by other investigators (Kipen et al., 1989; Townsend et al., 1978; Fishbeck et al., 1978; Tsai et al., 1983; Collins et al., 1991). However, this study has not appeared in the published literature and it conflicts with other published studies cited above.

**Source:** Deutsche Shell Chemie GmbH Eschborn

German rapporteur

**Flag:** Risk Assessment

13-SEP-2000

(1200)

**Remark:**

Most benzene epidemiology studies use exposure metrics such as cumulative exposure (the product of intensity [concentration] and time), yet little data are available that evaluate the adequacy of cumulative exposure as an exposure metric. This paper uses physiologically-based pharmacokinetic modelling (PBPK) to determine if benzene exposure intermittency or exposure peaks are important determinates of internal metabolite concentrations. The PBPK model used has five tissue groups, including the liver, fat, bone marrow, muscle, and an organ group. Typical

benzene exposures encountered in the petroleum distribution environment were used in the modelling. Based on a 19.2 mg/m<sup>3</sup> (3 ppm) exposure for 3 hours (9 ppm-hours) and then breaking the 9 ppm-hours into progressively shorter duration episodes, the model predicted that bone marrow metabolites were the same for the more discontinuous pattern. Varying concentration and time from low-level continuous exposures (19.2 mg/m<sup>3</sup> [3 ppm] for 6 hours) to higher, shorter duration exposures (up to 153.6 mg/m<sup>3</sup> [48 ppm] for 22.5 minutes), the level of total bone marrow metabolites for higher intensity versus longer duration exposures were the same. The authors conclude that, for typical petroleum distribution environments, the contribution of concentration and duration of exposure are approximately equal. In addition, short (within-a-day) discontinuities in exposure do not play a role in predicting total bone marrow metabolite concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1022)

**Remark:**

Most epidemiologic studies of benzene exposure examine risk associated with cumulative exposure (concentration X time). To further explore the possible impact of exposure rate effects on leukenogenesis, the authors used the Pliofilm data to examine effects of specific benzene long-term

average (LTA) exposure concentrations, rather than the estimated cumulative exposure of benzene. A dose rate effect is defined as an effect which primarily relies on exposure concentration, above that which would be predicted by the simple mathematical product of concentration X time. The three sets of LTA exposure estimates developed by previous authors (Rinsky et al., 1981; Crump & Allen, 1984; Paustenbach et al., 1992) were used in the study. Subsets of Pliofilm employees always exposed to less than or equal to specific concentration of benzene were examined. The results suggest that (a) AMML risk is shown only above a critical concentration of benzene exposure, measured as a long term average and experienced for years, (b) the critical concentration is between 160 and 192 mg/m<sup>3</sup> (50 and 60 ppm) using exposure estimates from three previous exposure assessments, (c) using the lowest exposure estimates (Rinsky et al., 1981), there was no suggestion of excess risk for workers who experienced LTA concentrations over their career of 64 mg/m<sup>3</sup> (20 ppm) or below, and (d) risks for total leukaemia are driven by risks for AMML, suggesting that AMML is the cell type related to benzene exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn (1023)  
06-JAN-1997

**Remark:** This study of 33,815 men, who first started work in the rubber industry between 1 January 1946 and 31 December 1960, followed their mortality experience up to 31 December 1975 to ascertain the number of deaths attributable to malignant disease and to compare these with the expected number calculated from the published mortality rates applicable to the male population of England and Wales and Scotland. No quantitative estimates of benzene exposures were available. A statistically significant excess of both lung and stomach cancer mortality was observed. A small excess of oesophageal cancer was also observed in both the tire and general rubber goods manufacturing sectors.

**Source:** Deutsche Shell Chemie GmbH Eschborn (870)  
06-JAN-1997

**Remark:** This is a prospective cohort study of approximately 11,000 male and 600 female employees in the Australian petroleum industry who had worked at least 5 years in 1981-1983. Jobs were coded according to classifications developed by the American Petroleum Institute to assess potential exposure. However, there were no quantitative exposure data available on benzene or other exposures; potential exposures were ranked on a qualitative scale from one to four. Deaths from lympho-hematopoietic cancers (LHC) were greater than expected based on the Australian national population. (Standardized Mortality Ratio [SMR] = 138; 95% Confidence Interval [CI] = 63-263). Incident cancers in this category were significantly elevated (Standardized Incidence Ratio [SIR] = 204; 95% CI = 125-315). All leukemia cell types

were approximately 1.5 to 4-fold elevated, but none were statistically significant. There was an apparent exposure-response trend for all LHC and qualitative measures of total hydrocarbon exposure, but this was not statistically significant ( $p = 0.6$ ). Analyses by workplace showed airports had the largest incidence excess (SIR = 1006; 95% CI = 208-2940), but this was based only on 3 cases. The largest LHC excesses were observed among workers first employed in the early 1960's. Definitive conclusions about the role of benzene and other exposures are not possible due to the lack of quantitative exposure data.

**Source:** Deutsche Shell Chemie GmbH Eschborn (204)  
06-JAN-1997

**Remark:** This study describes a case-control study of nine different causes of death, including leukemia, nested within a single cohort of 6678 male rubber workers. Each workers' job was classified into an occupational title grouping with functionally similar jobs and exposures. No quantitative exposure data for any exposures, including benzene, were available. Lymphatic leukemias were found to be most strongly associated with solvent-exposure areas, especially inspection, finishing, and repair. However, the presence of multiple chemical and physical exposures, the large number of different jobs held by most workers, and the lack of quantitative exposure data preclude any definitive conclusions regarding benzene or other exposures.

**Source:** Deutsche Shell Chemie GmbH Eschborn (755)  
06-JAN-1997

**Remark:** This document reviews previous concentration/effect assessments for benzene and develops a scientific basis for an air quality standard (AQS) on benzene. The review findings indicate that most previous assessments have been done using total leukemia mortality in relation to cumulative exposure. A linear dose-response model assuming no threshold is the most common model used. The review concludes these assumptions likely overestimate the effects associated with low level benzene exposures. The document concludes that an AQS of 30 parts per billion as an annual average is scientifically justified. This AQS is based on acute myelocytic leukemia, which a review of the available evidence suggests a no observed adverse effect level of 42 ppm for 1.3 years and a lowest observed adverse effect level of 39 ppm for 16 years for this endpoint. Several adjustment factors to account for sensitive subpopulations and other factors were used.

**Source:** Deutsche Shell Chemie GmbH Eschborn (232)  
06-JAN-1997

**Remark:** This is the unpublished report from a cytogenetic evaluation of peripheral lymphocytes from 52 workers exposed to low levels of benzene (less than 32 mg/m<sup>3</sup> [10 ppm]) at three benzene production facilities in Texas. The authors report a dose-related increase in the frequency of chromosome breaks and markers (combined) for exposures under 3.2 mg/m<sup>3</sup> (10 ppm), relative to a 44-person comparison group seen for preemployment examination. Statistically significant differences were found in the distribution of specific types of chromosomal aberrations. However, abnormal cells and chromatid breaks were not affected.

**Source:** Deutsche Shell Chemie GmbH Eschborn (893)  
06-JAN-1997

**Remark:** This is the published version of a study by Picciano (1978) which evaluated cytogenetic parameters among peripheral lymphocytes from 52 workers exposed to low levels (less than 32 mg/m<sup>3</sup> [10 ppm]) of benzene at three benzene production facilities in Texas. The findings reported are similar to the unpublished report (Picciano, 1978). However, the dose-related increase findings described in the unpublished report (Piccano, 1978) were deleted from the published version, and it was stated that the findings of increased aberration rates as compared to a 44 person comparison group were simply due to exposure below 32 mg/m<sup>3</sup> (10 ppm). Moreover, Killian and Daniel (1978) reported that peaks of 320 mg/m<sup>3</sup> (100 ppm) and above were previously encountered by the exposed workers. The EPA evaluated the information and concluded that it was not possible to determine whether the study related to recent concentrations below 32 mg/m<sup>3</sup> (10 ppm), or whether past concentrations up to 320 mg/m<sup>3</sup> (100 ppm) were involved. Other weaknesses of the study include scoring of cytogenetic findings by the author which may have resulted in a lack of blinding about exposure; a much lower frequency of all indices (breaks, gaps, etc.) than in a previous report (Benge et al., undated), which subsumed the study workers; a lack of a control for smoking; an imbalance in the age of the exposed versus control workers (26 versus 39); and concurrent exposures to other aromatic hydrocarbons. The fact that chromosome-type breaks and marker chromosomes were found in excess, but the total number of abnormal cells was similar is also inconsistent.

**Source:** Deutsche Shell Chemie GmbH Eschborn (894)  
06-JAN-1997

**Remark:** The authors report two analyses of workers exposed to low benzene concentrations. In the first analysis, 32 workers exposed to TWA concentrations of < 2.9 mg/m<sup>3</sup> (< 0.9 ppm) [but up to 2.6 mg/m<sup>3</sup> (0.8 ppm)] were compared to two groups of controls consisting of 27 and 39 workers. The study was blinded, controlled for age, gender, and smoking, and scored only 100 cells per individual. When compared to one group of controls, a statistically significant excess of chromosome breaks, gaps, and exchanges (combined) was found.

When compared to the second control group, the excess was not statistically significant. The first control group appears to have a very low frequency of the combined indices (including zero values). The second analysis involved workers who were exposed to slightly higher levels [0.4 mg/m<sup>3</sup>, up to 18.6 mg/m<sup>3</sup> (0.14 ppm, up to 5.8 ppm)]. This time, 200 rather than 100 cells were scored which reduces the statistical variation in the indices. The authors reported no excesses in the combined index (breaks, gaps and exchanges), nor SCE. The first result may have been due to random statistical variation since only 100 cells were scored, and control values showed considerable variation.

**Source:** Deutsche Shell Chemie GmbH Eschborn (290)  
06-JAN-1997

**Remark:** A total of 716 newly diagnosed acute nonlymphoblastic leukemia (ANLL) patients were evaluated prospectively to determine if karyotype is an independent prognostic factor for ANLL. Of the 716 patients, 660 had de novo ANLL, and 56 had secondary ANLL. Cases were grouped into two karyotype classifications, and both methods, especially the Chicago Classification, resulted in groups of patients with de novo ANLL with significantly different presenting clinical and hematologic features. Deletions of chromosomes 5 and 7 were observed in 4.2% and 4.4% of the de novo cases, respectively, and simultaneously in 3.2% of cases. Deletions of all or part of chromosomes 5 and/or 7 are the earliest clonal alterations detected in the development of myelodysplastic syndrome and acute myelogenous leukemia. The authors suggest this study is evidence that karyotype is an independent prognostic factor for ANLL.

**Source:** Deutsche Shell Chemie GmbH Eschborn (119)  
06-JAN-1997

**Remark:** Development of myelodysplasia (MDS) with subsequent progression to acute myeloid leukemia (AML) is an example of the multistep process of malignant transformation in which each step often relates to genetic abnormalities that can be directly seen as chromosomal aberrations. Therapy-related

MDS and AML (t-MDS and t-AML) may serve as an ideal model for a study of the genetic evolution of MDS and AML because chromosomal abnormalities are observed in most cases and because the disease is often diagnosed early due to a close patient follow-up. The cytogenetic characteristics at diagnosis were studied in 137 consecutive cases of t-MDS and t-AML, including 22 new cases, and correlated with the clinical characteristics and the course of the disease. Balanced translocations to chromosome bands 11q23 and 21q22 represent primary steps in pathways leading directly to overt t-AML. Specific chromosomal deletions or losses, on the other hand, represent primary or secondary events in alternative pathways leading to t-MDS with potential for subsequent transformation to overt t-AML. Loss of a whole chromosome 7 (-7) or deletion of its long arm (7q-) and deletion of the long arm of a chromosome 5 (5q-) were the most frequent primary abnormalities significantly related to t-MDS. Loss of a whole chromosome 5 (-5) was also a primary event, but surprisingly, was observed equally in t-MDS and in t-AML. Deletion of chromosome 13, including bands q13q14, was another less common primary aberration of t-MDS. Except for -7 and del(13q), these primary aberrations were most often observed together with secondary abnormalities. These included balanced aberrations involving band 3q26 and various deletions of chromosome 3, a gain of a whole chromosome 8, deletions of the short arm or loss of chromosomes 12 and 17, loss of a whole chromosome 18, and deletions of the short arm of chromosome 21. Deletions or loss of chromosomes 5 and 7 were significantly associated with previous therapy with alkylating agents ( $P = .002$ ), and balanced translocations to chromosome bands 3q26, 11q23, and 21q22 were significantly associated with previous therapy with drugs targeting DNA-topoisomerase II ( $P < .00005$ ). Other characteristic aberrations were not related to any specific type of therapy. The molecular changes believed to contribute to the development of t-MDS and t-AML have been identified for many of these chromosomal abnormalities. 1995 by The American Society of Hematology. Deutsche Shell Chemie GmbH Eschborn

**Source:**  
06-JAN-1997

(879)

**Remark:**

This letter-to-the-editor describes a small study of the effects of benzene and other solvent exposure on T-lymphocyte counts. The results showed decreased T-lymphocyte counts in 106 workers exposed to benzene, toluene, and xylene over 55 months. Benzene exposures were reported to be between 0 and approximately 384 mg/m<sup>3</sup> (0 and 120 ppm). It was not possible to evaluate whether this study addressed issues such as selecting an appropriate control population (n = 38) and blinding (evaluating the data without bias from knowledge of whether the individual was from the control or exposed group) in this brief

publication.  
**Source:** Deutsche Shell Chemie GmbH Eschborn (797)  
06-JAN-1997

**Remark:** This is a report from the Commission of the European Communities which develops a scientifically based occupational exposure limit for benzene. Presented in this report are preliminary results of an unpublished study of the influence of low level benzene exposures on the blood cells (Van Damme et al., 1991). The study examined all employees at a petrochemical plant who were working at the end of 1988 and had at least 5 haematological examinations during 1967-1988. There were 484 employees with a total of 17,404 blood samples. Based on exposure data from 1978-1988, more of the workers were exposed to benzene levels higher than 6.4 mg/m<sup>3</sup> (2 ppm) since 1971. The results of this study suggest an increased prevalence of leucopenia (defined as < 4000 cells/uL) for exposures of 0.64 to 1.92 mg/m<sup>3</sup> (0.2 to 0.6 ppm) which is well below no-effect level estimates of 64 mg/m<sup>3</sup> (20 ppm) for WBC effects reported by other investigators, (Kipen et al., 1989; Townsend et al., 1978; Fishbeck et al., 1978; Tsai et al., 1983; Collins et al., 1991). However, this study has not appeared in the published literature and it conflicts with the other published studies cited above.

**Source:** Deutsche Shell Chemie GmbH Eschborn (179)  
06-JAN-1997

**Remark:** This is a letter-to-the-editor which suggests that Kipen's et al. (1991) conclusions regarding decreasing benzene exposures and increasing WBC and RBC counts were flawed because pre-employment data showed a similar increase in WBC and RBC counts during 1940-48. Hornung et al.'s (1991) regression analysis of pre-employment counts predicted an increase of 16 counts/month compared to the 33 counts/month observed in the Kipen et al. (1988) data. Hornung et al. (1991) commented that a plot of monthly RBC and WBC counts showed an upward shift between July and August, 1947. While Hornung et al. (1991) do not offer a reason for the shift, they call into question a benzene effect on the counts as reported by Kipen. Kipen et al. (1991) replied that they thought the trend in pre-employment WBC counts shown by Hornung et al. (1991) might have a low r<sup>2</sup> (which is a measure of the proportion of variance explained) value (they suggested 0.04), while r<sup>2</sup> for WBC counts found in the Kipen et al. (1988) study was 0.88. Kipen et al. (1991) argued that the negligible trend reported by Hornung et al. (1991) actually verified the contention that the decrease in WBC counts was not the result of gradual or sudden changes in laboratory techniques over the nine year span.

**Source:** Deutsche Shell Chemie GmbH Eschborn (523)  
06-JAN-1997

**Remark:** Repeated dose toxicity  
The authors studied over 17,000 peripheral blood counts (RBC, WBC, Hb), accumulated during surveillance from 1940 through 1975, from a cohort of 459 benzene-exposed workers. A job exposure matrix consisting of 493 jobs versus time period entries was constructed, 86 (17%) of which were filled with actual exposure monitoring information. All entries prior to 1947 (i.e., from 1939) were done through extrapolation of more recent measures. Linear regressions demonstrated significant decreases in white and red cell counts, as well as hemoglobin, for workers exposed during the 1940's, without persistent trends over the ensuing 25 years. Strongly positive correlations were observed between these blood count fluctuations and fluctuations in retrospective estimates of benzene exposures for this plant for the earlier periods of surveillance (mean estimated exposure 1940 to 1948, 240 mg/m<sup>3</sup> [75 ppm]), but not for later years (mean estimated exposure 1948 to 1975, 48-64 mg/m<sup>3</sup> [15 to 20 ppm]). Overall, the data suggest that exposures of 64 mg/m<sup>3</sup> (20 ppm) or less have no effect on WBC, RBC, or Hb.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

13-SEP-2000

(622)

**Remark:** Fifteen degassers were acutely exposed over several days to high concentrations (192 mg/m<sup>3</sup> [> 60 ppm]) of benzene during removal of residual fuel (degassing) from shipboard fuel tanks. Eleven of the workers (73%) reported neurotoxic symptoms while degassing. Workers with more than 2 days (16 hours) of acute exposure were significantly more likely to report dizziness and nausea than those with 2 or fewer days of acute exposure. Repeated laboratory analyses performed over a 4-month period after the acute exposure revealed at least one hematologic abnormality consistent with benzene exposure in 9 (60%) of these degassers. However, there were no controls, and the effects on blood elements were not severe or consistent in the 15 workers. One year later, 6 workers (40%) had persistent abnormalities; an additional worker with normal hematologic parameters at the time of the initial evaluation subsequently developed an abnormality consistent with benzene exposure. Numerous large granular lymphocytes were observed on 6 (40%) of the peripheral blood smears. Despite these laboratory findings, there were no significant associations between the presence of hematologic abnormalities and either the number of hours of acute benzene exposure or the duration of employment as a degasser.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(774)

**Remark:** Repeated dose toxicity  
This study, which updates and expands the Yin et al. (1987) study, follows-up 74,828 benzene-exposed and 35,805 non-exposed workers in China. Pathology reports, medical records, and/or histopathologic material were reviewed for all patients with hematopoietic malignancies to ensure correct classification and to provide clinicopathologic descriptions. Eighty-two patients with hematopoietic neoplasms and related disorders were identified among benzene-exposed workers, including 32 cases of acute leukemia, 7--myelodysplastic syndrome (MDS), 9--chronic granulocytic leukemia (CGL), 20--malignant lymphoma or related disorder (ML), 9--aplastic anemia, and 5 others. Among the comparison group, 13 hematologic malignancies were observed, including 6 patients with acute leukemia, 2--CGL, 3--ML, and 2 others. The hematopathologic characteristics of the benzene-exposed ANLL cases resembled those following chemotherapy or radiotherapy. ANLL in workers exposed to benzene may represent a distinct clinicopathologic entity, with characteristics similar to treatment-related ANLL, including a preceding preleukemic phase in some patients. Results in our series, one of the largest to date, also indicate that a greater diversity of hematologic neoplasms is evident among benzene-exposed workers than previously described.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment  
13-SEP-2000 (1155)

**Remark:** Repeated dose toxicity  
The authors report 24 cases of aplastic anaemia in 508,818 Chinese workers either exposed to benzene or benzene-containing mixtures at estimated exposures up to 1035 mg/m<sup>3</sup> (333 ppm). Sixteen cases occurred in six provinces which equated to a prevalence of 3.5 per 100,000. The prevalence among more heavily exposed shoeworkers (12.1/100,000) was in excess of reported general population rates (1.5-2.4/100,000). Yin et al. (1987b) also reported on cases of leucopenia (< 4000 cells/mm<sup>3</sup>). The overall prevalence of leucopenia was 0.5%, with 0.94% in workers exposed to benzene, and 0.44% in workers exposed to benzene-containing mixtures. The highest prevalence was in the shoe-industry (1.25%) where benzene concentrations ranged from 0.06 to 844 mg/m<sup>3</sup> (0.02 to 264 ppm) with a median concentration of 40 mg/m<sup>3</sup> (12.5 ppm). Leucopenia cases were noted in work places where benzene concentrations were lower than 40 mg/m<sup>3</sup> (12.5 ppm), but it is unknown whether the prevalence of leucopenia was greater than expected in these work places.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment  
13-SEP-2000 (1299)

**Remark:**

Seventy-four adult patients with acute nonlymphocytic leukemia (ANLL) were classified retrospectively as to whether or not they had had occupational exposure to insecticides, chemicals and solvents, or petroleum products.

Fifty-eight patients were considered nonexposed and 16 were considered exposed. The chromosome banding pattern was abnormal in 37 of the 74 patients (50%). Twenty-five of the 58 (43%) nonexposed patients had a clonal chromosome abnormality, compared with 12 of the 16 (75%) exposed patients ( $p = 0.02$ ). Only 2 of 23 (8.7%) females with an abnormal karyotype were exposed, whereas 10 of 14 (71%) males with an abnormal karyotype were exposed. Either a  $-5/5q-$  or a  $-7/7q-$  was present in 87% of the exposed patients with a chromosome abnormality, compared to 28% of the aneuploid nonexposed patients. The  $-7/7q-$  abnormality was present in 7 of the 12 (58.3%) exposed patients, versus 5 of the 25 (20%) nonexposed patients with abnormal karyotypes ( $p < 0.05$ ). The  $-5/5q-$  anomaly was observed in 4 of the 12 (33%) exposed patients and in 4 of the 25 (18%) nonexposed abnormal patients. Our study supports the observations that a subset of patients with ANLL de novo have a history of occupational exposure and a unique pattern of chromosome abnormalities.

**Source:**

06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(427)

**Remark:**

The chromosome banding pattern of bone marrow cells and clinical findings, including cytologic diagnosis, response to therapy, and survival time, were compared in two groups of adult patients with acute nonlymphocytic leukemia (ANLL):

23 patients occupationally exposed to chemical solvents, insecticides, and petrol products and 33 patients with no history of occupational exposure to potential mutagenic/carcinogenic agents. As regards clinical findings, cases classified as AML were more common in the exposed group, whereas the monocytic varieties of ANLL were more common in the nonexposed group; neither the complete remission rate nor the median survival differed significantly. In both groups patients with only abnormal metaphases had poorer prognoses than those with normal bone marrow than those with normal bone marrow metaphases only. The detailed karyotypic findings showed striking differences between the two groups: (1) in the nonexposed group only 24.2% had chromosome aberrations, whereas 82.6% of the exposed patients had aberrations; (2) in the exposed group 84.2% of the patients with aberrations had at least one of four particular changes--monosomy 5 or 7 or trisomy 8 or 21, but in the nonexposed group none of the patients had monosomy 5 or trisomy 8, and only one patient had monosomy 7 and one had trisomy 21. None of the remaining aberrations seen in the nonexposed group were found in any of the exposed individuals.

**Source:** Deutsche Shell Chemie GmbH Eschborn (785)  
06-JAN-1997

**Remark:** In order to analyze the correlation between environmental exposure and the clinicopathological picture in acute myeloid leukemia (AML), cytogenetic, cyto-immunologic and clinical studies were performed in 70 newly diagnosed AML patients, 30 of which were anamnesticly exposed to pesticides (21 cases) or to organic solvents (9 cases). Clonal chromosome aberrations, with involvement of chromosome 5 and/or 7 were more frequently encountered among exposed patients. While the classical t(15;17), t(8;21) and t(9;11) were detected more frequently among non-exposed patients, other recurring chromosome changes in the exposed group were: rearrangements leading to total or partial monosomy 17p (5 cases), structural aberrations involving the band 16q22 (4 cases), trisomy 11q (2 cases), breaks involving bands 6p23, 7p14, 11q13 (2 cases each). Cytologically, trilineage myelodysplasia was observed in 21 exposed patients, whereas morphologic aberrations of the nonblast cell population were confined to a minority of cells in most patients non-exposed. Immunologic studies revealed positivity for the CD34 stem cell marker in 80% exposed patients vs 22% in the non-exposed group. Conventional chemotherapy achieved complete remission in 3/21 patients exposed and in 16/32 patients non-exposed. Median survival was 2 months in the former group and 9 months in the latter group. These findings show that AML following occupational exposure to pesticides and organic solvents may represent a distinct cytogenetic and clinicopathological entity.

**Source:** Deutsche Shell Chemie GmbH Eschborn (266)  
06-JAN-1997

**Remark:** Erfahrungen beim Menschen  
The authors review the data cited by OSHA in its final standard for exposure to benzene and conclude there is no clear scientific basis for a short-term-exposure limit (STEL). They argue that while leukemia and bone marrow toxicity were related to cumulative exposures of benzene received by workers, no evidence was presented that the rate of exposure at a given cumulative exposure contributed to the effects. Likewise, animal experiments suggested that exposures of several hours duration at a given level of benzene induced more bone-marrow toxicity when administered 3 rather than 5 days/week but did not indicate that the rate of exposure over shorter time scales played any role. The toxicokinetics of benzene in humans were also studied using a physiologically-based pharmacokinetic model to determine whether nonlinear dose-rate effects would be likely to result from peak exposures associated with an exposure dose of 8 ppm-hr, which is allowed under the permissible exposure limit. This led to three conclusions. First, the concentration of benzene in the bone marrow

should be sufficiently damped that the impact of a peak exposure should be minimal. Second, the peak concentration of benzene in the liver should be within the capacity of the cytochrome P450-system to maintain first-order metabolism. And finally, the maximum blood concentration of metabolites should be well below levels which have been shown to induce toxic effects in vitro. Taken together, the toxicokinetic relationships and the absence of clear experimental dose-rate effects suggest that the current STEL for benzene is unwarranted, assuming that 8-hr average exposures are kept below 1 ppm. While the argument can be made, on the basis of health considerations, that the existing 8-hr limit for benzene is too high, the rate of exposure during short periods appears to be irrelevant.

**Source:** Deutsche Shell Chemie GmbH Eschborn; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(876)

**Remark:**

Cytogenetic assays in peripheral blood lymphocytes (PBL) have been used extensively to survey the exposure of humans to genotoxic agents. The conceptual basis for this has been the hypothesis that the extent of genetic damage in PBL reflects critical events for carcinogenic processes in target tissues. Until now, no follow-up studies have been performed to assess the predictive value of these methods for subsequent cancer risk. In an ongoing Nordic cohort study of cancer incidence, 3182 subjects were examined between 1970 and 1988 for chromosomal aberrations (CA), sister chromatid exchange or micronuclei in PBL. In order to standardize for the interlaboratory variation, the results were trichotomized for each laboratory into three strata: low (1-33 percentile), medium (34-66 percentile), or high (67-100 percentile). In this second follow-up, a total of 85 cancers were diagnosed during the observation period (1970-1991). There was no significant trend in the standardized incidence ratio with the frequencies of sister chromatid exchange or micronuclei, but the data for these parameters are still too limited to allow firm conclusions. There was a statistically significant linear trend ( $P = 0.0009$ ) in CA strata with regard to subsequent cancer risk. The point estimates of the standardized incidence ratio in the three CA strata were 0.9, 0.7, and 2.1, respectively. However, the findings are based on a relatively limited number of cases, and other factors (e.g. genetic constitution, different occupational and lifestyle exposures) may have influenced the findings.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(465)

**Remark:**

Historically, investigations of causality of chronic diseases and occupational exposures have relied upon employment within an industry and/or job as a surrogate for exposure. Similarly, for investigations of dose-response relationships, length of employment within these categories

has been used. These surrogates, however, may result in large amounts of misclassification of subjects by exposure categories, which may severely affect risk estimates, particularly if the risks are low. Examples from the literature are provided to demonstrate that these surrogates for exposures may lower estimates of disease risks, obscure etiologic agents, create large confidence intervals (thereby reducing the likelihood of finding a statistical association), and affect dose-response relationships. Recently, more investigators have developed semiquantitative assessments, i.e., assigning jobs to low, medium, and high exposure categories. Although this approach is more satisfactory than the historical approach, it is less than satisfactory because the quantitative relationships among the categories are not known.

Incorrect

weighing of exposure categories can also result in misclassification of subjects when calculating measures such

as cumulative exposure. Quantitative assessment, i.e., assigning a value in units used in industrial hygiene monitoring, is ideally the best approach. However, such an approach may be difficult, if not impossible, because monitoring data are rarely sufficient to allow calculation of measured exposure levels. Thus, assessments often require judgment in assigning exposure level, which can also

lead to misclassification. Nevertheless, investigators should use the most quantitative procedure possible so as to

develop exposure estimates that are reflective of dose.

This approach will enhance the power of epidemiologic studies to detect and evaluate exposure-response associations.

**Source:**  
06-JAN-1997

Deutsche Shell Chemie GmbH Eschborn

(1088)

**Remark:**

This unpublished report is a cytogenetic evaluation of 290 benzene-exposed workers in a medical monitoring program from 1965-1975. Medical histories were taken on all workers prior to employment to obtain data on factors that might influence cytogenetic factors. Cytogenetic aberrations studied included chromatid breaks, chromosome breaks, dicentric, rings, and exchanges, and the proportion of abnormal cells. A total of 15 workers had sustained acute benzene exposures. Benzene exposures were estimated to be below 160 mg/m<sup>3</sup> (50 ppm) 8 hour-TWA prior to 1972 and well below 32 mg/m<sup>3</sup> (10 ppm) from 1973 to present. The comparison group comprised 973 pre-employment examinees who were deemed to have negligible exposure to chromosome-breaking agents. The results showed rates of chromosomal abnormalities were not increased among exposed workers compared with controls. There was no difference in the distribution of abnormal cell levels between exposed and control groups.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(93)

**Remark:** This is a textbook on occupational epidemiology. Chapters are devoted to epidemiologic study design, conduct, analysis, and interpretation. Examples of concepts are illustrated with examples from the occupational epidemiology literature.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(791)

**Remark:** This document presents results of a re-evaluation of the carcinogenic potency of benzene. The authors develop a mathematical model, consistent with a two-stage theory of carcinogenesis, that takes variable exposure and latency period into account. This model evolved by substituting in a sequential manner more plausible and scientifically defensible assumptions than those originally employed by EPA(1985). Two factors have the most pronounced effect upon the alteration of the unit risk estimate. These are the use of new statistical methods, primarily estimation techniques, and the requirement that two molecules of a benzene metabolite are needed to cause the transforming event. Since exposure for the pliofilm cohort was most probably underestimated and strong empirical and theoretical evidence exists suggesting that the relationship between benzene metabolites and the cell transforming event is non-linear, the linear model should be viewed as a highly conservative upper bound. The authors also present evidence that very distant benzene exposures (e.g. > 30 years before disease) may be irrelevant in determining risk.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1129)

**Remark:** Chromosome changes are studied in 21 cases of chronic benzene poisoning (avg 6 years exposure; range 1-28 years), both hypodiploid and hyperdiploid cells are significantly increased. Controls comprised 20 healthy subjects. Among the hypodiploid cells, deletion of Group C, E and G chromosomes is observed. Analysis of hyperdiploid cells reveals extra Group C and E chromosomes. The chromatid gaps and breaks are mostly found in poisoned cases, but fragments and minutes are less common. However, the finding of aneuploidy may not indicate that aneuploidy is a sensitive effect indicator of benzene because some subjects had simultaneous or previous benzene haemopathy.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(300)

**Remark:** This paper reviews data from studies done by the author to determine if benzene is leukemogenic in man. The author believes benzene is a human leukemogen based on the following data: (1) The incidence of leukemia in shoeworkers exposed to benzene in a period of 8 years in Istanbul was 13.6/100,000, which is significantly higher than that for leukemia in the general population. (2) Following the phase-out of benzene in Istanbul, the number of leukemic workers decreased and none were reported in the subsequent 3 years. (3) The development of leukemia in pancytopenic patients with benzene exposure was observed in 13 out of 51 patients. (4) The differences in the distribution of the types of leukemia in individuals exposed and in nonexposed groups were as follows: acute leukemia 96.1% in the former group, and 46% in the latter group. The high percentages of acute erythroleukemia and preleukemia were other interesting findings in the exposed group. (5) Two cases of leukemia were observed in a 6-year period at a tire cord manufacturing plant with 550 workers.

At one location in the plant the concentration of benzene measured by gas chromatography was nearly 110 ppm. Additionally, the author has studied 12 cases of malignant lymphoma, four cases of multiple myeloma, and six cases of lung cancer, all of whom were chronically exposed to benzene.

The possible role of benzene in the etiology of these malignancies is discussed.

**Source:** Deutsche Shell Chemie GmbH Eschborn (21)  
06-JAN-1997

**Remark:** This is a textbook dealing with the epidemiology of leukemia. The epidemiology of the four major leukemia cell types -- acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), and chronic myelocytic leukemia (CML) -- is reviewed.

The four major cell types show marked differences in risk factors and, by inference, have different etiologies. For example, ALL shows a distinct incidence peak in childhood, CML is related to the Philadelphia chromosome anomaly, and AML is the most frequent form of secondary leukaemia related to exposure to chemotherapeutic drugs and radiation treatment. CLL is the only subtype which has not been shown to be radiation-related. Furthermore, each leukaemic subtype is likely to have different target cells. The book also reviews genetic factors and physical and chemical agents associated with each major cell type.

**Source:** Deutsche Shell Chemie GmbH Eschborn (689)  
06-JAN-1997

**Remark:** Repeated dose toxicity  
This unpublished document reviews the benzene health effects literature to develop a leukemia medical surveillance program for benzene-exposed workers. The author reviews the hematologic effects of benzene exposure and the studies of leukemia incidence and mortality among benzene-exposed workers. Early U.S. literature on case reports of benzene exposure indicate 880 cases of cytopenia or pancytopenia, and 101 cases of aplastic anemia. Exposures in most cases were estimated to exceed 320 mg/m<sup>3</sup> (100 ppm) based upon general impressions of workplace exposure guidelines and practices during the time that exposures occurred. The literature review also suggested a fatal outcome in 13% of aplastic anemia cases and an 85% fatal outcome for idiopathic aplastic anemia.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur

**Flag:** Risk Assessment

13-SEP-2000 (578)

**Remark:** To study the correlation of environmental exposure to potentially mutagenic agents and the clinicopathologic picture in acute myeloid leukemia (AML), clinical features, morphologic characteristics, immunophenotype, and cytogenetics were studied in 59 patients with newly diagnosed AML. Based on interviews on occupational hazards and hobbies showing prolonged contact with pesticides (18 patients) and organic solvents (7 patients), 25 patients were categorized as "exposed." Light microscopic studies showed myelodysplasia involving multiple cell lineages in all assessable patients with professional exposure to pesticides and organic solvents, whereas morphologic aberrations of the non-blast cell population were confined to a minority of cells in unexposed patients. These findings were confirmed by electron microscopic studies in 31 patients. Immunologic analysis showed the presence of a minor megakaryoblastic component in six exposed patients and showed positive findings for the CD34 stem cell marker in 85% of exposed patients, a figure significantly higher as compared with that for unexposed subjects. Cytogenetic studies confirmed the frequent occurrence of 5q and/or 7q aberrations in patients occupationally exposed (10 of 25 cases). Other recurring chromosome aberrations in the exposed group were 17p, trisomy 11q, and translocation of 16q, 6p, 7p, and 11 p, whereas the classic AML-specific translocations (i.e., t[15;17]; t[8;21]) were detected only in unexposed subjects. Taken together, these findings document that AML in patients professionally exposed to toxic substances may represent a distinct cytogenetic and clinicopathologic entity. The clinicobiologic characteristics in these exposed patients are similar to the features of AML arising in patients with prior chemotherapy for another tumor, thus suggesting that

similar transformation pathways may underlie leukemogenesis induced by cytotoxic drugs and by environmental exposure to some pesticides or organic solvents.

**Source:** Deutsche Shell Chemie GmbH Eschborn (343)  
06-JAN-1997

**Remark:** Repeated dose toxicity  
This study examined 28 Korean workers with leucopenia, anaemia, or both from a population of 119 unspecified industrial workers. No haematologic abnormalities were reported for workers between 32 and 64 mg/m<sup>3</sup> (10 and 20 ppm). The author implied a threshold of 32 mg/m<sup>3</sup> (10 ppm) for cytopenia. There is a lack of information concerning the basis for the reported exposure concentrations.

This study was taken as an estimate of the NOAEL for cytopenia.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German rapporteur  
**Flag:** Risk Assessment  
13-SEP-2000 (183)

**Remark:** The authors report on a study which examined mutations of the red blood cell glycoprotein A protein in human bone marrow cells. Variant cells were classified on the basis of the mutation expressed, i.e., gene duplication versus point mutations or small deletions. A highly significant difference between 23 Chinese benzene workers exposed to a median of 96 mg/m<sup>3</sup> (30 ppm) benzene and 22 age and sex-matched unexposed controls was found for the gene duplicating (NN) genotype. No such difference was found for the genotype (N0) associated with point mutations or small deletions. Furthermore, lifetime cumulative benzene exposure was associated with the NN, but not the N0 genotype. The results indicate a propensity for benzene to cause gene-duplicating mutations characterised by large deletions or chromosomal re-arrangements. All subjects also showed evidence for haematotoxicity as they had reduced blood levels of all major blood constituents; thus, the glycoprotein A mutations may not be the most sensitive indicator of a benzene-induced effect.

**Source:** Deutsche Shell Chemie GmbH Eschborn (966)  
06-JAN-1997

**Remark:** ABSTRACT: Background. Except for the leukemogenic effects of benzene, there is inadequate or sparse evidence on the carcinogenicity of the most common monocyclic aromatic hydrocarbons. The purpose of this study was to generate hypotheses on associations between exposure to benzene, toluene, xylene, and styrene and various common types of cancer. Methods. In the context of a population-based case-control study carried out in Montreal, 3,730 cancer patients (15 types of cancers, not including leukemia) and

533 population controls were interviewed, and their job histories were translated by a team of experts into occupational exposures, including benzene, toluene, xylene, and styrene. In the present analysis, exposure to these substances was compared between each case series and a control group pooling selected cancer patients and population controls, using logistic regression analysis. Results. Exposure levels were low for most exposed subjects, and there was a high correlation between exposure to benzene, toluene and xylene. For most sites of cancer there was no evidence of excess risk due to these substances. However, limited evidence of increased risk was found for the following associations: esophagus-toluene, colon-xylene, rectum-toluene, rectum-xylene and rectum-styrene. Conclusions. These latter observations warrant further investigation.

ADDITIONAL INFORMATION: This is a population-based case-control study. Between 1979 and 1986, 4576 eligible cases were ascertained among men 35-70 years of age living in metropolitan Montreal. A total of 3730 (82%) eligible cases participated in the study. Case identification is likely to be complete given all hospitals in the Montreal area participated in the study. Male controls were selected from electoral lists and age stratified to the age distribution of cases. 533 (71%) of the 740 controls selected participated. Three types of control groups were used in the analysis: cancer controls, population controls, and a subset of cancer and population controls pooled together. As results were similar for all three control groups, the pooled group was used in the final analysis. Trained interviewers collected data on non-occupational factors (e.g., age, socioeconomic status, smoking) and lifetime, detailed work histories. A team of chemists and hygienists blinded to the study subjects' status translated each job history into a list of potential exposure. Qualitative exposure measures were derived and included estimated confidence that exposure actually occurred (possible, probable, definite), frequency of exposure during a normal workweek (<5% of the time, 5-30%, more than 30%), and concentration of agent (low, medium, or high - ranked relative to certain occupations). The sum of the product of duration, frequency, and concentration computed over each individual's work history was calculated to derive cumulative exposure. The CI index was then categorized as low, medium, and high as defined by cut points at the 70th and 90th percentiles of the distribution of all subjects. The analysis examined risk of cancer for four different chemicals (benzene, toluene, xylene, and styrene) and 15 different types of cancer (esophagus, stomach, colon, rectum, pancreas, lung, lung-oat cell, lung-squamous cell, lung-adenocarcinoma, prostate, bladder, kidney, melanoma,

non-Hodgkin's lymphoma, and Hodgkin's lymphoma). All unconditional logistic regression models included age, family income, a cumulative smoking index, ethnicity, smoking status, and respondent status (self or proxy). In addition, logistic models for lung cancer included variables for exposure to arsenic, asbestos, chromium VI, nickel crystalline silica, beryllium, cadmium, and polycyclic aromatic hydrocarbons. Bladder cancer models also included exposure for aromatic amines. For cancer sites with two or more substances having elevated OR, logistic regression models with all significant substances included in the model were performed (i.e, multiple exposure models). Exposure to toluene, benzene, and xylene was, for the majority of subjects, ranked as being certain exposure, medium frequency, and low concentration. Except for styrene, most subjects had their first exposure before 1950. There was a high degree of correlation between exposure to benzene, toluene, and xylene (58, 74, and 88% of subjects exposed to benzene, toluene, and xylene, respectively were exposed to all three substances). This high correlation made it difficult to disentangle the independent effects of each chemical. The percentage of all subjects who were exposed to benzene by duration of exposure was small (8.0% for 1-10 years, 3.7% for 11-20 years, and 7.1% for > 20 years). The odds ratios (OR) for virtually all benzene/cancer sites evaluated were near 1.0 and not statistically significant. The OR suggested by the authors to be suggestive of an association for benzene were as follows: colon/benzene (OR low exposure = 0.8; 95% confidence interval (CI)=0.6-1.2, OR medium exposure = 1.2; 95% CI=0.7-2.1, OR high exposure = 1.5; 95% CI=0.8-2.8) and rectum/benzene (OR low exposure = 1.3; 95% CI=0.9-2.0, OR medium exposure = 2.0; 95% CI=1.1-3.6, OR high exposure = 0.8; 95% CI=0.3-2.5). The colon/benzene and rectum/benzene associations were further investigated with multiple exposure logistic regression models; the results for both colon (OR low exposure = 0.6; 95% CI=0.4-0.9, OR medium & high exposure combined = 0.8; 95% CI=0.4-1.5) and rectum cancer (OR low = 1.2; 95% CI=0.5-2.8, OR medium & high exposure combined = 1.0; 95% CI=0.4-2.8) were at or near unity.

Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: Results of a case-control study in Montreal.

**Source:****Reliability:**

04-NOV-1998

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(403)

**Remark:**

ABSTRACT: Recent epidemiological data (Rushton et al., 1996, Schnatter et al., 1996) provide evidence of increased risk of leukemia in workers exposed to low levels of benzene (<1 ppm) and raised concern of necessity to reevaluate the national occupational exposure standard for benzene (1.6

ppm). As part of our R&D program for evaluation of cancer predictive values of biomarkers for genotoxic effects of occupational exposure to chemical human carcinogens, we performed cytogenetic studies in 114 Bulgarian workers from 4 different chemical plants, chronically exposed to benzene, and in a control group of 68 persons. Study subjects were divided into 4 groups: controls and subjects exposed to 1.1-1.7 ppm; 2.3-4.7 ppm and 7-24 ppm benzene. The cytogenetic biomarkers studied in peripheral lymphocytes (Ly) included chromosome aberrations (CA) and micronuclei, analyzed by the cytokinesis block (CB) MN assay. A significant 3 fold increase in the frequency of the percentage aberrant cells was found in workers of the lowest exposure group only ( $2.3 \pm 1.7$  vs  $0.67 \pm 0.7$  in controls) with an "exposure response" relationship. Age, smoking, and duration of exposure had no effect on the levels of CA. A highly significant 4-5 fold increase in the frequency of MN-binucleated Ly (BN-Ly) and MN in BN-Ly was found in all exposure groups. This increase was significantly correlated with age and duration of exposure in the medium exposure group. Based on the cytogenetic damage revealed in workers exposed to low levels of benzene (1.1-1.7 ppm) an increased carcinogenic risk was predicted and lowering of the national occupational exposure standard for benzene to 0.31 ppm was proposed. Cytogenetic biomarkers used in occupational surveillance programs: A report from ongoing Bulgarian cytogenic studies of workers exposed to benzene.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(4) not assignable

03-NOV-1998

(784)

**Remark:**

ABSTRACT: The purpose of the National Exposure Registry is to assess the long-term health consequences to a general population from long-term, low-level exposures to specific substances in the environment. This study investigates the health outcomes of 1,143 persons (1,127 living, 16 deceased) living in south central Texas who had documented environmental exposure to benzene (up to 66ppb) in tap water. As with all subregistries, face-to-face interviews were used to collect self-reported information for 25 general health status questions. Using computer-assisted telephone interviewing, the same health questions were asked 1 year (Follow-up 1, F1) and 2 years later (Followup 2, F2). The health outcome rates for Baseline and Followup 1 and 2 data collections for the Benzene Subregistry were compared with national norms, that is, the National Health Interview Survey (NHIS) rates. For at least one of the three reporting

periods, specific age and sex groups of the Benzene Subregistry population reported more adverse health outcomes when compared with the NHIS population, including anemia and other blood disorders, ulcers, gall bladder trouble, and stomach or intestinal problems, stroke, urinary tract disorders, skin rashes, diabetes, kidney disease, and respiratory allergies. Statistically significant deficits for the Benzene Subregistry population overall were found for asthma, emphysema, or chronic bronchitis; arthritis, rheumatism, or other joint disorders; hearing impairment; and speech impairment. No statistically significant differences between the two populations were seen for the outcomes hypertension; liver disease; mental retardation; or cancer. These results do not identify a causal relationship between benzene exposure and adverse health effects; however, they do reinforce the need for continued followup of registrants.

ADDITIONAL INFORMATION: This is a cross-sectional survey of self-reported health outcomes for 1143 individuals (1127 alive at interview, 573 males) living near a hazardous waste

site in Texas. All residents of the Three Lakes Municipal Utility District were targeted for inclusion, because benzene had been found in their groundwater (source of drinking water) at up to 66 ppb. All individuals were considered to be chronically exposed to benzene. No individual measurements were available and no questions relating to exposure modification (i.e water intake) appear to have been asked. Prior to the start of the study, extensive outreach was performed, including advanced publicity regarding the exposure and its health effects. Response rates were high, with more than 90% of contacted individuals being interviewed. All health outcomes were self-reported and were considered physician confirmed if the subject reported that a physician had told them the diagnosis. Physician confirmation was actually only attempted for cancer, and only 14 of 53 self-reported cancers were evaluated. Only 4 of the 14 self-reported cancer cases were reported correctly. Multiple comparisons were performed on subjects grouped by age, sex, and outcome.

Cancer analyses were based only on the 6 cancer deaths that had been correctly reported (out of 10 cancer deaths reported). Biological plausibility was inferred if any laboratory or epidemiological study suggested any impact on the organs of interest, regardless of the dose.

The National Exposure Registry: analyses of health outcomes from the benzene subregistry.

**Source:**  
**Reliability:**  
03-NOV-1998

EXXON Biomedical Sciences East Millstone, NJ  
(3) invalid

(169)

**Remark:**

ABSTRACT: OBJECTIVES: To assess the association between petrochemical exposure and spontaneous abortion, a retrospective epidemiological study in a large petrochemical complex in Beijing, China was conducted. METHODS: Plant employment records identified 3105 women who were married, were 20-44 years of age, and had never smoked. Of those, 3070 women (98.8%) reported at least one pregnancy. From this group, 2853 (93%) of the women participated in the study. According to their plant employment record, about 57% of these women workers reported occupational exposure to petrochemicals during the first trimester of their pregnancy. Trained interviewers administered a standardised questionnaire to this group of women and their husbands, collecting information on reproductive history, pregnancy outcomes, employment history, occupational exposure, smoking habits, alcohol consumption, indoor air pollution, and demographic variables. The results from the womens' first pregnancies were analyzed. RESULTS: There was a significantly increased risk of spontaneous abortion for women working in all of the production plants with frequent exposure to petrochemicals (8.8%; range of 5.8%-9.8%) compared with those working in nonchemical plants (2.2%; range of 0.0%-7.1%). Also, when a comparison was made between exposed and non-exposed groups within each plant, exposure to petrochemicals was consistently associated with an increased risk of spontaneous abortion. The overall odds ratio (OR) was 2.7 (95% confidence interval (95% CI) 1.8 to 3.9) after adjusting for potential confounders. When the analysis was performed with the exposure information obtained from the women' interview responses for (self reported) exposures, the estimated OR for spontaneous abortions was 2.9 (95% CI 2.0 to 4.0). The analysis was repeated by excluding those 452 women who provided inconsistent reports between recalled exposure and work history, and a comparable risk of spontaneous abortion (OR 2.9; 95% CI 2.0 to 4.4) was found. In analyses for exposure to specific chemicals, an increased risk of spontaneous abortion was found with exposure to most chemicals, and the results for benzene (OR 2.5; 95% CI 1.7 to 3.7), gasoline (OR 1.8; 95% CI 1.1 to 2.9), and hydrogen sulphide (OR 2.3; 95% CI 1.2 to 4.4) were significant. CONCLUSION: An increased risk of spontaneous abortion was found associated with the exposure to petrochemicals, including benzene, gasoline, and hydrogen sulphide.

ADDITIONAL INFORMATION: This is essentially a cross sectional survey, because previous reproductive history and exposure/job history was obtained during the interview. All exposure/job and pregnancy outcome information were self-reported. Information on exposure was solicited for each trimester of each first pregnancy and categorized into none, low, medium, and high based on perceived intensity. Association of petrochemical exposure with spontaneous

abortion.  
**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (2) valid with restrictions  
02-NOV-1998 (1288)

**Remark:** Re: Benzene and the dose-related incidence of hematologic neoplasms in China [letter; comment].  
Summary (no abstract): A study by Hayes et al of Chinese workers exposed to benzene and other chemicals noted an increased NHL risk of 3.0 (95% CI 0.9-10.5). In this letter to the editor, Otto Wong presents additional unpublished results from two earlier published studies; a chemical plant mortality study and a rubber manufacture mortality (Pliofilm) study. These earlier studies had not identified NHL cases specifically. In the reanalysis of the chemical workers study, there were 7 cases of NHL, with 5.12 expected (SMR 1.37 95% CI 0.55-2.82). In the rubber study, there were 3 NHL deaths and 3.28 expected (SMR 0.91 95% CI 0.19-2.66). He suggests that these results, as well as those from the published literature support no effect on NHL from benzene exposure.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (4) not assignable  
02-NOV-1998 (1273)

**Remark:** ABSTRACT: Objectives: The role of occupational exposures in hairy cell leukaemia was investigated through a multicentre, hospital based, case-control study. This paper analyses the role of exposure to solvents other than benzene in hairy cell leukaemia. Methods: The study included 226 male cases and 425 matched controls. Exposure to solvents was evaluated by expert case by case review of the detailed data on occupational exposures generated by specific interviews. Also, exposure to solvents was evaluated with an independently constructed job exposure matrix (JEM). Results: No association was found between hairy cell leukaemia and previous employment in a job exposed to solvents (odds ratio (OR) 0.9 95% confidence interval (95% CI) 0.6 to 1.3). ORs for the main occupational tasks exposed to solvents were around 1 and did not increase with the frequency or the duration of the tasks. No specific type of paint or glue was found to be significantly associated with hairy cell leukaemia. No association was found with exposure to solvents, taken as a whole, with either expert assessments or the JEM. No association was found with aromatic, chlorinated, or oxygenated subgroups of solvents. The ORs did not increase with the average intensity of exposure assessed by the experts, with the frequency of

use,  
or with the duration of exposure. Finally, no association was found with non-occupational exposure to solvents. Conclusions: The study did not show any association between exposure to solvents and hairy cell leukaemia.

ADDITIONAL INFORMATION: There were 378 cases identified, 278 of these were living (60%), 100 dead, and 50 non respondent. There were 809 eligible controls, with 425 completing questionnaires (57%). Controls were matched to cases on age, sex, admission date, and residence. Cases and controls had similar demographic characteristics. All subjects were sent self-administered questionnaires, but those who indicated solvent exposure were also administered semi-structured interviews by OH physicians. Exposures were assessed for intensity and duration. Conditional logistic regression was used to generate multivariate models. Occupational exposure to solvents and hairy cell leukaemia. EXXON Biomedical Sciences East Millstone, NJ (2) valid with restrictions (216)

**Source:**  
**Reliability:**  
02-NOV-1998

**Remark:** ABSTRACT: Objectives. A case-referent study was conducted to test the hypothesis that exposure to motor vehicle exhaust increases the risk of childhood cancer. Methods. Data from a study of residential magnetic field exposure and childhood cancer were used. From a population of 127,000 children living within 300 m of transmission lines in Sweden, 142 cases of childhood cancer were identified, including 39 cases of leukemia and 33 cases of central nervous system tumor. Approximately 4 referents per case were selected at random from the study base. The nitrogen dioxide content of the outdoor air was estimated as an indicator of motor vehicle exhaust. The applied methods give the 99th percentile of the nitrogen dioxide content of the outdoor air for 1-h averages over 1 year. Results. A relative risk estimate of 2.7 [95% confidence interval (95% CI) 0.9-8.5] was found for total cancer at exposure levels of  $\geq 50$  ug/m<sup>3</sup>, related to those with  $\leq 39$  ug/m<sup>3</sup>. At  $\geq 80$  ug/m<sup>3</sup>, the relative risk was estimated at 3.8 (95% CI 1.2-12.1). Elevated, but imprecise risk estimates were found for leukemia and central nervous system tumors. Conclusions. The results indicate an association between childhood cancer and motor vehicle exhaust, although the number of cases was small. These findings and the results of previous studies suggest that further studies of the association between motor vehicle exhaust and childhood cancer are warranted.

ADDITIONAL INFORMATION: Benzene was not measured directly, although the authors mention it as one component of auto exhaust. The study evaluated exposure to NO<sub>2</sub> as an estimate of exposure to auto exhaust. However, NO<sub>2</sub> was estimated from a model that included a background NO<sub>2</sub> and characteristics like traffic density, street width, and others. No questionnaires were administered, so covariates were limited to age, sex, power line exposure, SES, and geographic location. Exposure levels were defined by quartiles, but estimates below the median were used as the reference category. Although no explanation was given for this disproportionate assignment, one suspects that a monotonic trend would not have resulted from a strict assignment by quartiles. Indeed, most of the excess risk was in the highest exposure quartile. A total of over 140 cases of childhood cancer were identified, but results were reported only for leukemia (39 cases) and CNS cancer (33 cases). Exposure to motor vehicle exhaust and childhood cancer. EXXON Biomedical Sciences East Millstone, NJ

**Source:****Reliability:**

03-NOV-1998

(2) valid with restrictions

(356)

**Remark:**

ABSTRACT: In this article, investigators report on the presence and nature of chemical sensitivities and other indices of illness in a cohort of workers excavating a new subway tunnel located under a former gasoline station. The workers were exposed to gasoline fumes for up to approximately 2 months when they inadvertently dug into soil contaminated by gasoline. The cohort was unique in several ways: (a) contact with gasoline was made by the workers at a time when no one had complained of multiple chemical sensitivities syndrome; (b) all were males of low socioeconomic status; (c) the exposure was well documented; (d) the cohort could be considered 'naive' because, at the time of the study, the men were not members of support groups and were not being seen by clinical ecologists, and they were not labeled, either by self or others, as having multiple chemical sensitivities syndrome or any related diagnosis; and (e) at the time of interview, all workers we contacted appeared to be either gainfully employed or laid off temporarily and seeking gainful employment. We explored the health status of the workers at two different times: (1) soon after the tunnel was closed as a result of high, measured benzene-exposure levels and (2) 10-13 months after the tunnel was closed. The workers were chronically overexposed to gasoline fumes, after which approximately one-fourth (26.7%) of our random sample of relatively naive, low-socioeconomic-status male laborers -- although neither disabled nor generally litigious --- reported the new onset of chemical hypersensitivities and other characteristics

that fit conservative criteria for multiple chemical sensitivities syndrome.

ADDITIONAL INFORMATION: The design of the study is closest to a clinical experiment, i.e. treatment and control groups, but without any randomization. There were 10 men in the tunnel group and approximately 20 each in the general population and MCS groups. No statistical tests are done. The test groups differ on basic socio-demographic factors. Self-reported symptoms represent the outcomes. Development of multiple chemical sensitivities in laborers after acute gasoline fume exposure in an underground tunneling operation.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(3) invalid

03-NOV-1998

(277)

**Remark:**

Benzene exposure, glutathione S-transferase theta homozygous deletion, and sister chromatid exchanges. Recent studies have shown a strong positive correlation between chromosomal aberrations and future cancer risk. Sister chromatid exchange (SCE) has been widely applied in monitoring early biological effects to assess human genetic risk of cancer at the population level. We studied 45 Chinese workers (23 in the painting workshop of a glass factory with occupational exposure to benzene, and 22 fitters and planers in the punching and planing machine workshops of a nearby shipyard without such an exposure) to examine the association between occupational exposure to benzene and SCE frequency in peripheral blood lymphocytes. We also sought to investigate whether the glutathione S-transferase class theta gene (GSTT1) affects individual susceptibility to cytogenetic damage induced by in vivo exposure to benzene or in vitro exposure to diepoxybutane. The time-weighted average concentrations of benzene were 0.71 ppm in the exposed group and 0.03 ppm in the non-exposed group. Controlling for age, gender and educational level, cigarette smoking was significantly associated with increased SCE frequencies ( $P < 0.05$ ), while GSTT1 genotype was significantly associated with DEB-induced SCEs ( $P < 0.01$ ). There was no relationship between benzene exposure and baseline or DEB-induced SCEs. After stratification by smoking status, the GSTT1 deletion was a significant predictor of DEB-induced SCEs for both smokers ( $P < 0.05$ ) and nonsmokers ( $P < 0.01$ ). A significant benzene-GSTT1 interaction was found in nonsmokers ( $P < 0.05$ ). Our study suggests that GSTT1 is an important determinant of heterogeneity in individual susceptibility to chromosomal damage associated with exposure to benzene.

Although the biological significance of SCE formation is unclear, such exchanges are considered a sensitive

indication of chromosomal damage.

The induction of SCE is a sensitive endpoint for certain chemical exposures, and could serve as a biomarker for early biological effects in evaluating human cancer risk. However, detection of DNA adducts by using antibodies appears more specific than SCE analysis.

In this study occupational benzene exposure was found not to be associated with either baseline SCEs or DEB-induced SCEs.

The present study revealed a suggestive but nonsignificant gene-environment interaction between the GSTT1 genotype and occupational benzene exposure among women.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

04-NOV-1998

(1289)

**Remark:**

Benzene exposure in car mechanics and road tanker drivers  
The purpose of this study was to identify professional factors related to benzene exposure and to deduce suitable safety measures. Atmospheric benzene, urinary muconic acid (tt-MA) and leukocyte alkaline phosphatase activity (LAPA) were evaluated among 66 car mechanics, 34 road tanker drivers, and 28 nonexposed workers. Professional and medical questionnaires were filled in at the same time. Atmospheric benzene was significantly higher among road tanker drivers than among car mechanics. The arithmetic mean  $\pm$  SD, median, and geometric mean values were, respectively, 0.48  $\pm$  1.49, 0.14, and 0.06 mg/m<sup>3</sup> among car mechanics and 1.88  $\pm$  4.18, 0.68, and 0.65 mg/m<sup>3</sup> among road tanker drivers. In the latter case the increase was caused by transport of unleaded petrol and correlated with the volume of the tank. Among car mechanics, tobacco smoking, windy conditions, dismantling of petrol filters, and handling of petrol increased atmospheric benzene levels. Urinary muconic acid was increased significantly among car mechanics (148  $\pm$  137, 127, and 111  $\mu$ -g/g) and among road tanker drivers (309  $\pm$  420, 137, and 151  $\mu$ -g/g) as compared with the controls (49  $\pm$  46, 33, and 33  $\mu$ -g/g). Among road tanker drivers, alcohol intake and transportation of unleaded petrol increased the excretion of muconic acid, which was also directly related to the volume of the tank. Among car mechanics, professional factors (dismantling of petrol filters, handling of and washing of hands with petrol) and nonprofessional factors (tobacco smoking and damaged skin on the hands and forearms)

increased muconic acid excretion. In the control group, tobacco smoking increased its excretion. LAPA was not significantly modified among exposed workers. There was a weak but significant linear correlation between LAPA and muconic acid. These results suggest that to reduce exposure to benzene in unleaded petrol, individual and collective safety measures should be imposed in both occupations.

**Source:**  
**Reliability:**  
04-NOV-1998

EXXON Biomedical Sciences East Millstone, NJ  
(2) valid with restrictions

(580)

**Remark:**

Chromosome aberrations in peripheral blood lymphocytes have been used for many years to monitor human populations exposed to potential carcinogens. Recent reports have confirmed the validity of this approach by demonstrating that elevated levels of chromosome aberrations in lymphocytes are associated with subsequent increased cancer risk, especially for increased mortality from hematological malignancies including acute myeloid leukemia (AML). We postulated that this approach could be improved in two ways:

(a) by detecting oncogenic disease-specific aberrations; and

(b) by using chromosome painting so that many more metaphases could be analyzed. Numerical and structural aberrations in chromosomes 8 and 21 are commonly observed in

AML. In the present study, we painted chromosomes 8 and 21 in lymphocyte metaphases from 43 healthy workers exposed to benzene, an established cause of AML, and from 44 matched controls. To examine dose-response relationships the workers

were divided into two groups at the median exposure level, a

lower-exposed group ( $\leq 31$  ppm;  $n = 21$ ), and a higher-exposed group ( $> 31$  ppm;  $n = 22$ ). Benzene exposure was associated with significant increases in hyperdiploidy of chromosomes 8 (1.2, 1.5, and 2.4 per 100 metaphases;  $P < 0.0001$ ) and 21 (0.9, 1.1, and 1.9 per 100 metaphases;  $P < 0.0001$ ). Translocations between chromosomes 8 and 21 were increased up to 15-fold in highly exposed workers (0.01, 0.04, and 0.16 per 100 metaphases;  $P < 0.0001$ ). In one highly exposed individual, these translocations were reciprocal and were detectable by reverse transcriptase-PCR.

These data indicate a potential role for  $t(8;21)$  in benzene-induced leukemogenesis and are consistent with the hypothesis that detection of specific chromosome aberrations

may be a powerful approach to identify populations at increased risk of leukemia.

Increased Translocations and Aneusomy in Chromosomes 8 and 21 Among Workers Exposed to Benzene

**Source:**  
**Reliability:**  
04-NOV-1998

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(1054)

**Remark:**

Interleukin 1 alpha and hematological examination in mechanics exposed to low benzene concentrations  
OBJECT: To examine the hypothesis of Renz and Kalf relative to the involvement of interleukin 1 alpha (IL-1 alpha) in the development of anemia in benzene-exposed workers. According to this hypothesis, benzene inhibits the cleavage of the IL-1 alpha precursor (proIL-1 alpha) to mature IL-1 alpha and the lack of this cytokine is responsible for benzene-induced bone marrow suppression. This inhibition of the processing of proIL-1 alpha is attributed to an inhibition of calpain. METHOD: Selection of a population of mechanics exposed to low levels of benzene from fuels, assessment of usual exposure and lifetime exposure duration, and measurements of concentrations of workplace-air benzene and urinary benzene metabolites. Determination of IL-1 alpha concentrations was done by a whole-blood assay after lipopolysaccharide stimulation and a hematological examination was carried out. Statistical analysis considered several possible confounding factors, particularly smoking and drinking habits. DESIGN: Cross-sectional study. RESULTS: The level of exposure of the mechanics to benzene from fuels was mostly well below 1 ppm. IL-1 alpha production was not decreased in mechanics exposed to benzene from fuels, and no correlation between IL-1 alpha concentrations and red blood cell counts appeared. With the exception of a slight decrease in red blood cell counts in mechanics, no hint of a toxic effect of exposure on hematological parameters was found. CONCLUSIONS: The hypothesis of Renz and Kalf could not be confirmed. Although the low level exposure of the study population and methodological factors are possible explanations, it cannot be excluded that the hypothesis of Renz and Kalf is not generalizable to benzene-exposed humans. Presently, one cannot advise the measurement of IL-1 alpha production for biological effect monitoring of workers exposed to low concentrations of benzene.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

04-NOV-1998

(525)

**Remark:**

Evaluation of personal exposure to monoaromatic hydrocarbons  
OBJECTIVES: To evaluate the personal exposure of members of the general public to atmospheric benzene, toluene, and the xylenes, excluding exposure from active smoking. METHOD: 50 volunteers were equipped with active air samplers for direct measurement of personal exposure to monoaromatic hydrocarbons (MAH) and an activity diary was completed during each sampling period. Exposures were also estimated indirectly by combining activity data with independent

measurements of hydrocarbon concentrations in several microenvironments. RESULTS: Personal exposure were generally well in excess of those which would be inferred from outdoor measurements from an urban background monitoring station. A wide range of sources contribute to exposure, with indoor and in car concentrations generally exceeding those measured at background outdoor locations. Environments contaminated with tobacco smoke were among those exhibiting the highest concentrations. Personal exposures determined indirectly from activity diaries/microenvironment measurements were well correlated with those determined directly with personal samplers. Personal 12 hour daytime exposures to benzene ranged from 0.23-88.6 ppb (mean 3.81 ppb), with 12 hour night time exposures of 0.61-5.67 ppb (mean 1.94 ppb) compared with an annual average concentration of 1.18 ppb at the nearest suburban fixed site monitoring station. The excess of personal exposure over fixed site concentrations was greater for benzene and toluene than for the xylenes. CONCLUSION: A wide range of sources contribute to personal exposures to monoaromatic hydrocarbons with exposure duration being as important a determinant of total exposure as concentrations. Exposures generally exceed those estimated from concentrations measured by background fixed point monitors. Microenvironment sampling combined with activity diary information can provide satisfactory estimates of personal exposure to these compounds.

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(683)

**Remark:**

Benzene and total hydrocarbon exposures in the upstream petroleum oil and gas industry. Occupational exposures to benzene and total hydrocarbons (THC) in the Canadian upstream petroleum industry are described in this article. A total of 1547 air samples taken by 5 oil companies in various sectors (i.e., conventional oil/gas, conventional gas, heavy oil processing, drilling and pipelines) were evaluated and summarized. The data includes personal long- and short-term samples and area long-term samples. The percentage of samples over the occupational exposure limit (OEL) of 3.2 mg/m<sup>3</sup> or one part per million for benzene, for personal long-term samples ranges from 0 to 0.7% in the different sectors, and area long-term samples range from 0 to 13%. For short-term personal samples, the exceedance for benzene is at 5% with respect to the OEL of 16 mg/m<sup>3</sup> or five parts per million in the conventional gas sector and none in the remaining sectors. THC levels were not available for all sectors and had limited data points in others. The percentage exceedance

of the OEL of 280 mg/m<sup>3</sup> or 100 parts per million for THC as gasoline ranged from 0 to 2.6% for personal long-term samples. It is recommended that certain operations such as glycol dehydrators be carefully monitored and that a task-based monitoring program be included along with the traditional long- and short-term personal exposure sampling.

**Source:**  
**Reliability:**  
07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(1211)

**Remark:**

An evaluation of modeled benzene exposure and dose estimates published in the Chinese-National Cancer Institute collaborative epidemiology studies. Risk estimates and cause and effect determinations are directly dependent on exposure and dose-response relationships. Recently, relative risks and excess cancer mortality attributed to occupational benzene exposure have been published in collaborative studies conducted by Chinese investigators and scientists from the National Cancer Institute. The results of these studies suggest increased risk of acute nonlymphocytic leukemia at relatively low benzene concentrations and associations with cancers not previously associated with benzene exposure. These studies are potentially important due to their size and potential to more thoroughly investigate the link between benzene exposure and cancer. However, there are questions concerning the validity of exposure and dose estimates supporting relative risk characterizations in these studies. Apparent discrepancies between modeled exposure and dose estimates and sources of actual measured exposure information and clinical markers of benzene toxicity raise serious concerns questioning the reliability of relative risk and cancer associations stated in these studies.

**Source:**  
**Reliability:**  
07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(166)

**Remark:**

A critique of the exposure assessment in the epidemiologic study of benzene-exposed workers in China conducted by the Chinese Academy of Preventive Medicine and the US National Cancer Institute. As reviewed in some detail in the present paper, workers employed in a wide variety of industries were included in the Chinese benzene study, and were exposed to not only benzene but also a wide range of other industrial chemicals. To attribute any or all health effects observed in the exposed cohort to benzene without examining other concomitant exposures is not appropriate. Although it was stated that one of the major objectives of the expanded

study was to examine the effects of other risk factors, no such examination was made in any of the analyses in the expanded CAPM-NCI study. The CAPM-NCI study suffered from a number of limitations. One of the most serious limitations of the study involved the exposure estimates developed by the US NCI team. Comparing the assumptions used in the development of estimates and the exposure estimates themselves to actual data reported previously by the Chinese investigators revealed numerous inconsistencies and, in many cases, large discrepancies. It appeared that the exposure estimates were consistently lower than the actual exposure data. The so-called indirect validation conducted by the NCI team served no useful purpose, since by definition it could not validate the absolute values of the estimates. NCI was fully aware of some of the inadequacies of its exposure estimates. Although in a 1994 paper, the NCI team recognized that little confidence could be attached to the estimated (e.g., only 2% of the estimates for the time interval 1949-1959 and only 6% of the estimates prior to 1975 were rated in the high confidence category), the inadequacy of the estimates was never mentioned or discussed in any subsequent analyses or in the latest report (Hayes et al., 1998). Instead, the exposure of the workers was hailed as "well characterized" (Hayes et al., 1998). In conclusion both CAPM and NCI have made substantial efforts in studying the relationship between benzene exposure and various malignancies. Unfortunately, there were many inherent problems in the data as well as serious limitations in the exposure estimates. Because of these unresolved problems and limitations, many of the results in the CAPM-NCI study are unreliable. Therefore, the conclusions of the study, particularly those involving exposure estimates, are not justified.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

07-JUL-2005

(1267)

**Remark:**

Non-Hodgkin's lymphoma and exposure to benzene in a multinational cohort of more than 308,000 petroleum workers, 1937 to 1996.

Petroleum workers are exposed to benzene or benzene-containing petroleum products. As such, studies of these workers provide an opportunity for investigating the relationship between benzene and non-Hodgkin's lymphoma (NHL). However, few cohort studies of petroleum workers report results of NHL separately. One reason is that NHL is usually grouped with other lymphopietic cancers in the analysis. Another reason is the relatively small number of NHL cases in some studies. To determine the risk of NHL in petroleum workers, we identified 26 cohorts of petroleum

workers in the United States, the United Kingdom, Canada, Australia, Italy, and Finland. Authors of the original studies were contacted, and data on the number of observed deaths and person-years of observation were requested. Data from these studies were reviewed individually as well as combined in a pooled analysis (meta-analysis). In particular, results for individual cohorts, most of which had never been reported before, were presented. The combined multinational cohort consisted of more than 308,000 petroleum workers (6.6 million person-years), and the observation period covered an interval of 60 years from 1937 to 1996. A total of 506 NHL deaths were observed, compared with 561.68 expected. The standardized mortality ratio was 0.90 and the 95% confidence interval was 0.82 to 0.98. Analyses were performed by type of facility and industrial process. Stratum-specific standardized mortality ratios (95% confidence intervals) were 0.96 (0.86 to 1.07) for US refinery workers, 1.12 (0.90 to 1.37) for non-US refinery workers, 0.64 (0.50 to 0.82) for product (gasoline) distribution workers, and 0.68 (0.47 to 0.95) for crude oil workers. When individual cohorts were stratified by length of observation, no pattern was detected. In general, exposure levels before 1950 were much higher than thereafter. However, analysis of workers by hire date (< 1950, > or = 1950) revealed no difference in NHL mortality. Furthermore, none of the individual studies showed significant exposure-response relations. In summary, results from individual studies, as well as from the pooled analysis, indicated that petroleum workers were not at an increased risk of NHL as a result of their exposure to benzene or other benzene-containing petroleum products in their work environment. This conclusion was supported by cohort studies of workers in other industries who were exposed to benzene as well as by population-based case-control studies of NHL and occupational exposures.

**Source:****Reliability:**

07-JUL-2005

(1) valid without restriction

(1269)

**Remark:**

Nested case-control study of leukaemia, multiple myeloma, and kidney cancer in a cohort of petroleum workers exposed to gasoline.

OBJECTIVES: This nested case-control study was based on data

in a cohort study of more than 18,000 petroleum distribution

workers exposed to gasoline, which contains about 2%-3%

benzene. Risks of leukaemia, acute myeloid leukaemia, multiple myeloma, and kidney cancer were examined relative to exposure to gasoline. METHODS: For each case, up to five individually matched controls were selected. Analyses based on the Mantel-Haenszel procedure as well as univariate and

multivariate conditional logistic regression were performed for each disease category. Jobs with similar exposures were grouped into homogeneous categories for analysis. Several quantitative indices of exposure to gasoline were used in the analyses: duration of exposure, cumulative exposure, frequency of peak exposure, and time of first exposure. RESULTS: No increased risks for the four cancers were found for any job category. Analyses with logistic regression models based on duration of exposure, cumulative exposure, and frequency of peak exposure did not show any increased risk or exposure-effect relation. Time of first exposure to gasoline was also found to be unrelated to the four diseases under investigation. CONCLUSION: Exposure to gasoline or benzene at the concentrations experienced by this cohort of distribution workers is not a risk factor for leukaemia (all cell types), acute myeloid leukaemia, multiple myeloma, or kidney cancer.

**Source:**  
**Reliability:**  
07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(1270)

**Remark:**

BACKGROUND: Benzene is a human leukemogen. Risk assessment, and the setting of occupational and environmental standards, has assumed that risk is constant in time after a unit of exposure. Leukemia risk is known to vary with time after exposure to ionizing radiation. METHODS: A matched case-control study of leukemia risk in relation to the temporal pattern of benzene exposures was performed using data from the National Institute of Occupational Safety and Health. RESULTS: Leukemia risk following exposure to benzene varied with time in a manner similar to that following exposure to ionizing radiation. More recent exposures were more strongly associated with risk than were more distant ones. There was no significant relation between leukemia death and benzene exposures incurred more than 20 years previously. CONCLUSIONS: Recent analyses of specific occupational and environmental carcinogens, including benzene and radon, have indicated that cancer risk tends to decline as the time from exposure increases. This suggests that standards for the control of occupational or public risk must be selected to control exposures over a narrower time frame than the usual lifetime one. In the case of benzene, it would appear that risk is attributable primarily to exposures incurred during the previous 10 to 20 years, with exposures in the most recent 10 years being the most potent. To limit risk, exposures must be controlled during that interval. It is important that epidemiologists explore the temporal pattern of risk in their studies to facilitate the risk assessment of other carcinogens. Leukemia after exposure to benzene: temporal trends and implications for standards.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005 (359)

**Remark:** Benzene and non-Hodgkin's lymphoma. Incidence rates for non-Hodgkin's lymphoma (NHL) have been rising throughout the world for several decades, and no convincing explanation exists for the majority of this increase. The commonest subtypes of NHL have no well-defined aetiological factors but lymphoma development has been linked with exposure to a variety of chemicals, including nitrates, pesticides, herbicides, and solvents. Benzene, a solvent and important constituent of petrochemical products, is a potent lymphomagen in experimental animals and high-dose exposure in humans is associated with both acute myeloid leukaemia and NHL. Much current interest centres on the possibility that environmental benzene exposure in the general public may underlie a proportion of the increase in NHL. Seventy per cent of benzene exposure in the environment is derived from vehicle exhaust emissions, whose increase has closely paralleled the rise in frequency of the disease. Mathematical modelling has been used to calculate an acceptable concentration of benzene in air based on risk estimates derived from industrial exposure, but the recommended target concentration in the U.K. of 1 ppb is regularly exceeded in urban locations. Detailed investigation of the health effects of low-level benzene exposure awaits an accurate assay for quantifying long-term human exposure. The  $(32)\text{P}$  post-labelling technique for the detection of toxin-specific DNA adducts is extremely sensitive and has been applied in the biomonitoring of exposure to a number of carcinogens, but benzene-DNA adducts have to date proved elusive of detection.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005 (848)

**Remark:** Cytogenetic changes in subjects occupationally exposed to benzene. Humans are exposed to benzene from various occupational and environmental sources. The genotoxic effects of benzene were assessed in peripheral blood lymphocytes of 36 workers employed in the shoe industry for a period extending from seven months to over 30 years. Chromosomal aberrations and sister chromatid exchanges were used as indicators of genotoxic effects. The incidence of dicentric chromosomes in the exposed group was significantly higher than in the control group ( $P < 0.05$ ). No significant increase was

detected between the working period in the exposed group and chromosomal aberrations. Sister chromatid exchange (SCE) frequency was not significantly increased in the exposed group.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005

(604)

**Remark:**

Benzene in blood as a biomarker of low level occupational exposure. The occupational airborne exposure to benzene of 150 workers employed in petrol stations and a refinery plant was assessed using personal sampling pumps. All workers provided blood samples after the end of work and on the following morning before resuming work. Benzene concentrations in the blood of 243 non-occupationally-exposed subjects were also measured. The median occupational benzene exposure for all 150 workers studied was 80 microgram/m<sup>3</sup>. Overall median blood benzene of all workers was 251 ng/l at the end of the shift, and 174 ng/l the following morning. The benzene concentrations measured in blood collected the following morning proved to be significantly lower than those measured at the end of the shift. Median blood benzene for the 243 'normal' subjects was 128 ng/l, which was significantly lower than that measured in the workers before a new work shift. The median blood benzene concentration was significantly higher in smokers than in non-smokers, both in the general population (210 ng/l vs. 110 ng/l) and in the exposed workers at the end of the shift (476 ng/l vs. 132 ng/l) and the following morning (360 ng/l vs. 99 ng/l). End-of-shift blood benzene correlated significantly with environmental exposure; this correlation was better in the 83 non-smokers than in the 67 smokers. In non-smokers with the median benzene occupational exposure of 50 microgram/m<sup>3</sup>, no difference was found in blood benzene concentration in exposed and non-exposed subjects.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005

(158)

**Remark:**

Leukaemia, lymphoma, and multiple myeloma in seamen on tankers. OBJECTIVES: To investigate the risk of lymphatic and haematopoietic malignancies in deck crew on tankers exposed to cargo vapours. METHODS: The study design was as a nested case-referent study in two cohorts of male Swedish seamen 20-64 years of age at the national census 1960 (n 13,449) and 1970 (n 11,290), respectively. Cases were detected by record linkage with the Swedish Cancer Register 1961-79 and

1971-87, respectively. For each case, three to five age matched referents from the population were selected. Exposure was assessed from data in the Swedish Registry of Seamen and from a register of Swedish ships. RESULTS: Seamen in the 1970 cohort, who had been exposed to cargo vapours for at least one month on chemical or product tankers, had an increased risk of lymphatic and haematopoietic malignancies (Mantel-Haenszel odds ratio (OR) 2.6, 95% confidence interval (95% CI) 1.1 to 5.9)) with a significant exposure-response relation (conditional logistic regression analysis,  $p = 0.04$ ). The ORs were increased for both lymphoma (3.2), multiple myeloma (4.0), and leukaemia (1.6), but the increase was only significant for non-Hodgkin's lymphoma (OR 3.3, 95% CI 1.1 to 10.6). There were no significantly increased risks for the 1960 cohort or for seamen exposed only on crude oil tankers, but these groups had few exposed cases and low cumulative exposure to benzene and other light petroleum products. CONCLUSIONS: Seamen exposed to cargo vapours from gasoline and other light petroleum products on chemical or product tankers had an increased incidence of lymphatic and haematopoietic malignancies. One possible cause is exposure to benzene during loading, unloading, and tank cleaning operations.

Additional Comments: The investigators of this study pointed out that the number of exposed cases ( $n=46$ ) was comparatively small and the results have to be interpreted with caution.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(2) valid with restrictions

07-JUL-2005

(832)

**Remark:**

Cigarette smoking is associated with an increased risk of leukemia; benzene, an established leukemogen, is present in cigarette smoke. By combining epidemiologic data on the health effects of smoking with risk assessment techniques for low-dose extrapolation, we assessed the proportion of smoking-induced total leukemia and acute myeloid leukemia (AML) attributable to the benzene in cigarette smoke. We fit both linear and quadratic models to data from two benzene-exposed occupational cohorts to estimate the leukemogenic potency of benzene. Using multiple-decrement life tables, we calculated lifetime risks of total leukemia and AML deaths for never, light, and heavy smokers. We repeated these calculations, removing the effect of benzene in cigarettes based on the estimated potencies. From these life tables we determined smoking-attributable risks and benzene-attributable risks. The ratio of the latter to the former constitutes the proportion of smoking-induced cases attributable to benzene. Based on linear potency models, the

benzene in cigarette smoke contributed from 8 to 48% of smoking-induced total leukemia deaths [95% upper confidence limit (UCL), 20-66%], and from 12 to 58% of smoking-induced AML deaths (95% UCL, 19-121%). The inclusion of a quadratic term yielded results that were comparable; however, potency models with only quadratic terms resulted in much lower attributable fractions--all < 1%. Thus, benzene is estimated to be responsible for approximately one-tenth to one-half of smoking-induced total leukemia mortality and up to three-fifths of smoking-related AML mortality. In contrast to theoretical arguments that linear models substantially overestimate low-dose risk, linear extrapolations from empirical data over a dose range of 10- to 100-fold resulted in plausible predictions.

The contribution of benzene to smoking-induced leukemia. EXXON Biomedical Sciences East Millstone, NJ

**Source:****Reliability:**

07-JUL-2005

(1) valid without restriction

(635)

**Remark:**

OBJECTIVES: Animal inhalation studies and theoretical models

suggest that the pattern of formation of benzene metabolites

changes as exposure to benzene increases. To determine if this occurs in humans, benzene metabolites in urine samples collected as part of a cross sectional study of occupationally exposed workers in Shanghai, China were measured. METHODS: With organic vapour monitoring badges, 38

subjects were monitored during their full workshift for inhalation exposure to benzene. The benzene urinary metabolites phenol, catechol, hydroquinone, and muconic acid were measured with an isotope dilution gas chromatography mass spectroscopy assay and strongly correlated with concentrations of benzene air. For the subgroup of workers (n = 27) with urinary phenol > 50 ng/g creatinine (above which phenol is considered to be a specific indicator of exposure to benzene), concentrations of each of the four metabolites were calculated as a ratio of the sum of the concentrations of all four metabolites (total metabolites) and were compared in workers exposed to > 25 ppm v < or = 25

ppm. RESULTS: The median, 8 hour time weighted average exposure to benzene was 25 ppm. Relative to the lower exposed workers, the ratio of phenol and catechol to total metabolites increased by 6.0% (p = 0.04) and 22.2% (p = 0.007), respectively, in the more highly exposed workers.

By contrast, the ratio of hydroquinone and muconic acid to total metabolites decreased by 18.8% (p = 0.04) and 26.7% (p = 0.006), respectively. Similar patterns were found when

metabolite ratios were analysed as a function of internal benzene dose (defined as total urinary benzene metabolites), although catechol showed a more complex, quadratic relation with increasing dose. CONCLUSIONS: These results, which are consistent with previous animal studies, show that the relative production of benzene metabolites is a function of exposure level. If the toxic benzene metabolites are assumed to be derived from hydroquinone, ring opened products, or both, these results suggests that the risk for adverse health outcomes due to exposure to benzene may have a supralinear relation with external dose, and that linear extrapolation of the toxic effects of benzene in highly exposed workers to lower levels of exposure may underestimate risk.

Urinary excretion of phenol, catechol, hydroquinone, and muconic acid by workers occupationally exposed to benzene.

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(964)

**Remark:**

BACKGROUND: The present study was conducted among Chinese workers employed in glue- and shoe-making factories who had an average daily personal benzene exposure of  $31 \pm 26$  ppm (mean  $\pm$  SD). The metabolites monitored were S-phenylmercapturic acid (S-PMA), trans, trans-muconic acid (t,t-MA), hydroquinone (HQ), catechol (CAT), 1,2,4-trihydroxybenzene (benzene triol, BT), and phenol.

METHODS: S-PMA, t,t-MA, HQ, CAT, and BT were quantified by HPLC-tandem mass spectrometry. Phenol was measured by GC-MS.

RESULTS: Levels of benzene metabolites (except BT) measured in urine samples collected from exposed workers at the end of workshift were significantly higher than those measured in unexposed subjects ( $P < 0.0001$ ). The large increases in urinary metabolites from before to after work strongly correlated with benzene exposure. Concentrations of these metabolites in urine samples collected from exposed workers before work were also significantly higher than those from unexposed subjects. The half-lives of S-PMA, t,t-MA, HQ, CAT, and phenol were estimated from a time course study to be 12.8, 13.7, 12.7, 15.0, and 16.3 h, respectively.

CONCLUSIONS: All metabolites, except BT, are good markers for benzene exposure at the observed levels; however, due to

their high background, HQ, CAT, and phenol may not distinguish unexposed subjects from workers exposed to benzene at low ambient levels. S-PMA and t,t-MA are the most

sensitive markers for low level benzene exposure.

Validation of biomarkers in humans exposed to benzene: urine metabolites.

**Source:****Reliability:**

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

07-JUL-2005

(914)

**Remark:**

Analysis of urinary S-phenylmercapturic acid and trans, trans-muconic acid as exposure biomarkers of benzene in petrochemical and industrial areas of Korea. OBJECTIVES: Recently, S-phenylmercapturic acid (S-PMA) and trans,trans-muconic acid (t,t-MA) in urine have been proposed as reliable biomarkers for monitoring occupational exposure to benzene. The aim of this study was to test the applicability of S-PMA and t,t-MA as exposure biomarkers and to monitor the occupational exposure level and the extent of environmental contamination from benzene in Korea. METHODS: The urinary excretion of S-PMA and t,t-MA in rats after the intraperitoneal administration of benzene (0.88-800 mg/kg body weight, 7 days) was examined. These biomarkers were also validated in human urine samples collected from elementary schoolchildren in several industrial areas including chemical manufacturing plants, oil refineries, and natural gas-producing installations in Korea. Urine was collected from elementary schoolchildren in a mountain village with no known occupational exposure to benzene and air pollution as the reference group. RESULTS: In rats, there was a significant relationship between the benzene concentration and the excretion of the urinary S-PMA and t,t-MA as a function of concentration, and the excretion of benzene metabolites peaked on the first day after intraperitoneal administration. In human urine, higher levels of S-PMA and t,t-MA were detected more frequently in petrochemical industrial areas than in areas with no known occupational exposure to benzene. CONCLUSIONS: These results show that the quantitative determination of S-PMA and t,t-MA in urine can be used as a reliable exposure biomarker for benzene, and they also suggest that extensive attention to benzene exposure is needed for maintaining the health of the population in Korea.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(344)

**Remark:**

A petrochemical worker with aplastic anemia was referred to our hospital. He worked in a petroleum resin-producing factory and had been exposed to low-level benzene while packaging the powder resin and pouring lime into a deactivation tank. According to the yearly environmental survey of the working area, the airborne benzene level was approximately 0.28 ppm. Exposure to benzene, a common chemical used widely in industry, may progressively lead to pancytopenia, aplastic anemia, and leukemia. The hematotoxicity of benzene is related to the amount and

duration of exposure. Most risk predictions for benzene exposures have been based on rubber workers who were exposed to high concentrations. In the petroleum industry, the concentration of benzene is relatively low, and there are disputes over the toxicity of low-level benzene because of a lack of evidence. In this paper we report the case of aplastic anemia induced by low-level benzene exposure.

Additional Comments: Concomitant exposures to other airborne chemicals or contaminants at the worker's factory were not examined and potential contribution due to other chemicals has not been ruled out.

Aplastic anemia in a petrochemical factory worker.

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(2) valid with restrictions

(63)

**Remark:**

OBJECTIVES: This study examined the relationship between risk of premenopausal breast cancer and occupational exposure to benzene and polycyclic aromatic hydrocarbons (PAH) and whether the proposed relationship between PAH and breast cancer differed by tumor estrogen receptor (ER) status. METHODS: In a case-referent study of premenopausal breast cancer, occupational histories and other information were obtained through interviews, and job-exposure matrices were used to assess exposure to PAH and benzene. RESULTS: A dose-response relationship for the probability of exposure to benzene [low: odds ratio (OR) 1.64, 95% confidence interval (95% CI) 0.64-4.21; high: OR 1.95, 95% CI 1.14-3.33) and to PAH (low: OR 1.56, 95% CI 0.78-3.12; high: OR 2.40, 95% CI 0.96-6.01). Risk increased with duration of exposure to benzene, but not to PAH. A dose-response relationship was not evident for the intensity of exposure to benzene or to PAH. When analyses were stratified by tumor ER status, PAH exposure was related to a greater increase in the risk of ER-positive (OR 2.27, 95% CI 1.14-4.54) than ER-negative (OR 1.12, 95% CI 0.47-2.64) breast cancer. Risk of ER-positive, but not ER-negative, tumors increased with the probability of exposure to PAH. CONCLUSIONS: The findings suggest an association between risk and occupational exposure to benzene. Although it was difficult to study PAH independently of benzene, there was some suggestion of an association between PAH exposure and ER-positive tumors. These data should be interpreted with caution because of the limitations of this study, including low-response rates and small numbers of exposed persons. Risk of premenopausal breast cancer in association with occupational exposure to polycyclic aromatic hydrocarbons and benzene.

**Source:****Reliability:**

EXXON Biomedical Sciences East Millstone, NJ

(2) valid with restrictions

07-JUL-2005

(886)

**Remark:**

Assessment of complete blood count variations among workers exposed to low levels of benzene. The effect of benzene on white blood cell and red blood cell counts, hemoglobin level, mean corpuscular volume (MCV), and platelet count was investigated among workers in a small petroleum company. The investigated cohort consisted of 105 workers exposed to low levels of benzene between 1967 and 1994. The average level of benzene exposure per year ranged between 0.14 parts per million and 2.08 parts per million (8-hour time-weighted average). The mean complete blood count (CBC) demonstrated values within normal ranges. With the exception of white blood cells, all other CBC values were significantly reduced during the follow-up period. Length of employment was significantly related to the changes in MCV and platelet counts. The reductions in MCV were significant only among workers who had been employed for more than 10 years at this particular company. The findings of this study suggest that low levels of benzene may affect CBC values. CBC values may serve as a useful tool for biological monitoring for workers with low-level benzene exposure.

Additional Comments: The investigators indicated that there was no consistent CBC by individual for each year of the study and that confounding factors such as smoking and other non-work related sources were not taken into account since no control group was used.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(2) valid with restrictions

07-JUL-2005

(616)

**Remark:**

Effect of benzene, toluene, xylene on the semen quality of exposed workers. The effect on semen and sperm quality of workers following short- and long-term exposure to benzene, toluene, and xylene was examined. Semen and blood of 24 married workers exposed to benzene, toluene, and xylene from shoe-making, spray painting, or paint manufacturing facilities were collected. Benzene, toluene, and xylene concentrations in blood and semen were determined using a headspace chromatographic method. Routine sperm tests were conducted and acrosin activity detected. Results showed that benzene, toluene, and xylene were in blood and semen of some ex-workers in an environment where air concentrations of benzene, toluene, and xylene exceeded the max. allowable concentration (MAC). This result was not observed in control

group workers. There were also some effects on the quality of semen in exposed workers. For example, the percentage of semen with liquefaction time, and the level of toluene in semen, and a neg. correlation between sperm vitality, sperm activity, or acrosin activity and working history. Results suggested this mixture could affect semen and sperm quality,

which might be the main reason of abnormal pregnancies among

wives of workers exposed to benzene, toluene, and xylene. Further studies are required to confirm these findings.

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(2) valid with restrictions

(1285)

**Remark:**

Although relatively rare, leukemias place a considerable financial burden on society and cause psychologic trauma to many families. Leukemia is the most common cancer in children. The causes of leukemia in adults and children are largely unknown, but occupational and environmental factors are strongly suspected. Genetic predisposition may also play

a major role. Our aim is to use molecular epidemiology and toxicology to find the cause of leukemia and develop biomarkers of leukemia risk. We have studied benzene as a model chemical leukemogen, and we have identified risk factors for susceptibility to benzene toxicity. Numerous studies have associated exposure to benzene with increased levels of chromosome aberrations in circulating lymphocytes of exposed workers. Increased levels of chromosome aberrations have, in turn, been correlated with a heightened

risk of cancer, especially for hematologic malignancy, in two recent cohort studies in Europe. Conventional chromosome

analysis is laborious, however, and requires highly trained personnel. Further, it lacks statistical power, as only a small number of cells can be examined. The recently developed fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based technologies have allowed the detection of specific chromosome aberrations. These techniques are far less time consuming and are more sensitive than classical chromosomal analysis. Because leukemias commonly show a variety of specific chromosome aberrations, detection of these aberrations by FISH and PCR in peripheral blood may provide improved biomarkers of leukemia risk.

Biomarkers of leukemia risk: benzene as a model.

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(1056)

**Remark:**

Benzene oxide (BO) reacts with cysteinyl residues in hemoglobin (Hb) and albumin (Alb) to form protein adducts (BO-Hb and BO-Alb), which are presumed to be specific biomarkers of exposure to benzene. We analyzed BO-Hb in 43 exposed workers and 42 unexposed controls, and BO-Alb in a subsample consisting of 19 workers and 19 controls from Shanghai, China, as part of a larger cross-sectional study of benzene biomarkers. The adducts were analyzed by gas chromatography-mass spectrometry following reaction of the protein with trifluoroacetic anhydride and methanesulfonic acid. When subjects were divided into controls (n = 42) and workers exposed to < or =31 (n = 21) and >31 p.p.m. (n = 22)

benzene, median BO-Hb levels were 32.0, 46.7 and 129 pmol/g globin, respectively (correlation with exposure: Spearman r = 0.67, P < 0.0001). To our knowledge, these results represent the first observation in humans that BO-Hb levels are significantly correlated with benzene exposure. Median BO-Alb levels in these 3 groups were 103 (n = 19), 351 (n = 7) and 2010 (n = 12) pmol/g Alb, respectively, also reflecting a significant correlation with exposure (Spearman

r = 0.90, P < 0.0001). The blood dose of BO predicted from both Hb and Alb adducts was very similar. These results clearly affirm the use of both Hb and Alb adducts of BO as biomarkers of exposure to high levels of benzene. As part of

our investigation of the background levels of BO-Hb and BO-Alb found in unexposed persons, we analyzed recombinant human Hb and Alb for BO adducts. Significant levels of both BO-Hb (19.7 pmol/g) and BO-Alb (41.9 pmol/g) were detected, suggesting that portions of the observed background adducts reflect an artifact of the assay, while other portions are indicative of either unknown exposures or endogenous production of adducts.

Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(1296)

**Remark:**

Benzene increases aneuploidy in the lymphocytes of exposed workers: a comparison of data obtained by fluorescence in situ hybridization in interphase and metaphase cells.

Benzene is an established human leukemogen that increases the level of chromosome aberrations in lymphocytes of exposed workers. Numerical aberrations (aneusomy) can be observed by fluorescence in situ hybridization (FISH) in both interphase and metaphase cells. Whereas interphase FISH

allows nondividing cells to be analyzed, one advantage of metaphase FISH is that it can also detect structural changes. The present study compares the abilities of metaphase and interphase FISH to detect aneusomy of chromosomes 7 and 8 in healthy benzene-exposed human

subjects. Metaphase and interphase cells from the peripheral blood of 43 workers exposed to benzene (median = 31 ppm, 8-hr TWA) and 44 frequency-matched controls were analyzed by FISH. Normal diploid cells contained two hybridization signals, whereas those that were potentially monosomic contained one, trisomic 3 and tetrasomic 4. The frequency of cells with one hybridization signal for chromosome 7 in metaphase spreads rose from  $2.72 \pm 0.19$  (% , mean  $\pm$  SE) in controls to  $3.79 \pm 0.63$  in workers exposed to 31 or fewer ppm benzene and  $5.9 \pm 0.85$  in those exposed to more than 31 ppm ( $P(\text{trend}) < 0.0001$ ). No similar dose-dependent increase in the frequency of cells with one hybridization signal was observed by interphase FISH, probably because of probe overlap artifact. Although significant dose-dependent increases in the frequency of cells with three hybridization signals for chromosome 7 were detected by both methods in the higher-exposed group, a larger, more significant difference was detected by metaphase FISH between controls and workers exposed to 31 or fewer ppm. Similar data were obtained for chromosome 8. Interphase and metaphase FISH were moderately correlated for three hybridization signals but not for one hybridization signal in chromosome 7 or 8. In general, metaphase FISH was more sensitive in detecting both monosomy and trisomy in the lymphocytes of exposed workers.

**Source:**  
**Reliability:**  
07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

(1308)

**Remark:**

Mortality and cancer incidence among Swedish paint industry workers with long-term exposure to organic solvents. The aim of this update on a cohort of male paint industry workers was to determine whether an excess of mortality and incidence of lymphatic and hematopoietic tumors, particularly multiple myeloma, still exists, and, if so, to determine if it is due to exposures occurring before the mid-1950s, when benzene disappeared as a solvent in the Swedish paint industry. METHODS: The cohort of 411 men who had worked in the Swedish paint industry and had been exposed to organic solvents for at least 5 years during 1955-1975 was followed from 1961 to 1994 for causes of death in the mortality register and from 1961 to 1992 for cases of cancer in the Swedish cancer register. RESULTS: The number of paint industry workers who had died, plus the number of deaths in the major disease groups and the number of cancers reported to the cancer registry, was close to the expected. The incidence of prostatic cancer increased somewhat [standardized incidence ratio (SIR) 1.5, 95% confidence interval (95% CI) 1.0-2.2]. Among the workers first

employed  
in 1956 or earlier, there was an increase in both the  
incidence and mortality from all lymphatic and  
hematopoietic  
tumors [SIR 2.3, 95% CI 1.0-2.2; standardized mortality  
ratio (SMR) 2.0, 95% CI 0.7-4.4]. The excess was  
particularly marked for multiple myeloma (SIR 3.8, 95% CI  
0.8-11; SMR 4.4, 95% CI 0.9-13). CONCLUSIONS: Employment in  
the Swedish paint industry before 1957 may have entailed  
some excess risk of lymphatic and hematopoietic tumors,  
particularly multiple myeloma. A significant excess of  
prostatic cancer was not linked to any particular  
employment  
period and deserves further investigation.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(711)

**Remark:**

A pharmacokinetic study of occupational and environmental  
benzene exposure with regard to gender.  
Using physiologically-based pharmacokinetic (PBPK)  
modeling,  
occupational, personal, and environmental benzene exposure  
scenarios are simulated for adult men and women. This  
research identifies differences in internal exposure due to  
physiological and biochemical gender differences.  
Physiological and chemical-specific model parameters were  
obtained from other studies reported in the literature and  
medical texts for the subjects of interest. Women were  
found  
to have a higher blood/air partition coefficient and  
maximum  
velocity of metabolism for benzene than men (the two most  
sensitive parameters affecting gender-specific  
differences).  
Additionally, women generally have a higher body fat  
percentage than men. These factors influence the internal  
exposure incurred by the subjects and should be considered  
when conducting a risk assessment. Results demonstrated  
that  
physicochemical gender differences result in women  
metabolizing 23-26% more benzene than men when subject to  
the same exposure scenario even though benzene blood  
concentration levels are generally higher in men. These  
results suggest that women may be at significantly higher  
risk for certain effects of benzene exposure. Thus,  
exposure  
standards based on data from male subjects may not be  
protective for the female population.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(155)

**Remark:**

A multicolour tandem-labelling fluorescence in situ hybridization (FISH) procedure was used to detect chromosome alterations in peripheral blood cells of a group of Estonian petrochemistry workers. Twelve workers employed in benzene production and five cokery workers, together with eight unexposed rural controls, were enrolled in the study. The methodology employed, based on the in situ hybridization of adjacent centromeric and pericentromeric regions, allowed the simultaneous detection of both chromosome breakage, involving damage-prone pericentromeric regions, and hyperploidy in interphase cells. Blood smears from all subjects were hybridized with chromosome 1 specific probes, in order to detect genotoxic damage in circulating lymphocytes and granulocytes. Moreover, lymphocyte cultures were established, harvested 48 h following mitogen stimulation and hybridized with the tandem chromosomes 1 and 9 probes. No significant difference in the incidence of breakage was detected in the nucleated cells of blood smears of exposed vs. control subjects. In contrast, modest but significantly increased frequencies of breakage affecting both chromosomes 1 and 9 were observed in the cultured lymphocytes of the benzene-exposed workers compared to the unexposed controls, suggesting an expression of premutagenic lesions during the S-phase in vitro. Across the entire study group, the frequencies of breakage affecting chromosomes 1 and 9 in the stimulated lymphocytes were highly intercorrelated ( $p < 0.001$ ). No significant difference was found in the incidence of hyperploidy among the study groups, although a tendency to higher values was observed in benzene-exposed workers. Although the relatively small size of the study groups does not allow firm conclusions on the role of occupational exposure, the observed patterns are suggestive of effects in the benzene-exposed workers. This work also shows that tandem labelling FISH can be usefully applied in human biomonitoring, allowing the simultaneous detection of both hyperploidy and chromosome breakage at interphase in different cell types.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(744)

**Remark:**

Premature centromere division (PCD, i.e., separation of centromeres during the pro-metaphase/metaphase of the mitotic cycle) seems to be a possible manifestation of chromosome instability in human chromosome-breakage syndromes. Chromosome instability also frequently occurs in

the peripheral blood lymphocytes (PBL) of humans occupationally exposed to clastogenic agents, and is considered an etiol. factor of neoplastic diseases. To investigate the importance of PCD in cancer risk assessment, the frequency of PCD in PBL of 400 Hungarian subjects was studied. The various groups comprised 188 control donors and 212 subjects occupationally exposed to different genotoxic chems., such as acrylonitrile (ACN) and/or DMF, benzene, cytostatic drugs, ethylene oxide (ETO), mixed exposure in the rubber industry, mixed organic solvents including CCl4, hot oil-mist, bitumen, and polychlorinated biphenyls (PCB). Data were compared with chromosomal aberration frequencies determined in the same samples. PCD yields were significantly higher in populations exposed to mixed chems., crude oil, and cytostatic drugs vs. controls. PCD involving >3 chromosomes were also more frequent in ETO- and oil mist-exposed groups than in others. Results indicated that PCD induction is neither incidental nor artificial. As a consequence, it is suggested that PCD be developed into a new, exposure-related cytogenetic biomarker to more adequately assess occupational cancer risks. A further follow-up epidemiol. and cytogenetic investigation of PCD is in progress.

The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals.

**Source:**  
**Reliability:**  
07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ  
(2) valid with restrictions

(729)

**Remark:**

The absorption of benzene through human skin. The article is the first English translation of the classical study by Hanke, Dutkiewicz, and Piotrowski on the percutaneous absorption of benzene in humans. Portions of the original Polish-language article entitled "The Absorption of Benzene through Human Skin " have appeared in many publications, and the article is one of the most commonly cited studies of benzene ever conducted. This research, despite its careful design and the precision of its method, could not be conducted today in most countries of the world. The use of human subjects, including the authors, in a study of skin absorption of a known carcinogen would not be allowed. Some might even object to the publication and citation of the study on ethical grounds. But the importance of this seminal study of the percutaneous absorption of benzene, the further studies it induced, and its influence over the eventual regulation of benzene exposure in the workplace, is undisputed.

**Source:**  
**Reliability:**

EXXON Biomedical Sciences East Millstone, NJ  
(1) valid without restriction

07-JUL-2005

(471)

**Remark:**

Benzene is an established cause of leukemia at high doses, but the risk it poses at exposures of <1 ppm in air is difficult to quantify. Molecular biomarkers may improve the accuracy of this risk assessment. We have therefore attempted to develop and validate biomarkers of exposure, early effect and susceptibility to benzene. We have shown that NQO1 genotype and cytochrome P4502E1 phenotype are important biomarkers of susceptibility. Further, since chromosomal aberrations (CA) have been shown to be predictive of future cancer risk and specific CA are found in leukemias, we have proposed that specific CA may be excellent biomarkers of early effect. Leukemia-specific CA were found at elevated rates in the blood of 43 workers exposed to high levels of benzene (median, 31ppm). A larger study is planned for the year 2000 that will use these validated markers in biological samples from a substantially larger number of workers exposed to lower levels of benzene.

The design of this new study will be presented together with

a summary of data generated from our original cross-sectional study in highly exposed workers.

Molecular biomarkers of benzene exposure and risk  
EXXON Biomedical Sciences East Millstone, NJ

**Source:****Reliability:**

07-JUL-2005

(4) not assignable

(1057)

**Remark:**

A Public Health Goal (PHG) of 0.00014 mg/L (0.14 ppb) is proposed for benzene in drinking water, and assumes a de minimis cancer risk level of 10<sup>-6</sup> from lifetime exposure to benzene. The PHG is based on observations of increased rates

of leukemia among two cohorts of workers exposed to benzene:

rubber hydrochloride workers in the U.S. (Paxton et al., 1994) and benzene-exposed workers from various industries in

China (Hayes et al., 1997). After evaluating the evidence regarding the expected shape of the dose-response curve at low doses, cancer potency estimates were calculated from the

cohort data using Poisson regression and linear relative risk models. Estimates of lifetime risk to the general population from constant exposure to benzene were calculated

using life table analyses. The pattern of leukemia risk following exposure to benzene was investigated and observed to be similar to the pattern of risk observed for radiation and chemotherapeutic agents. Accordingly, lifetime risk estimates were calculated such that exposures greater than 30 years in the past would not contribute significantly to leukemia risk. The best upper-bound estimates of lifetime

leukemia risk resulting from air exposures of the general population to benzene were similar for the U.S. rubber workers (0.044 ppm-1) and the Chinese workers (0.056 ppm-1).

The two risk estimates were combined and converted to a population-based cancer potency of 0.055 (mg/kg-d)-1 for inhalation exposures, which was scaled to 0.11 (mg/kg-d)-1 for oral exposures. Due to the shape of the dose-response curves and other considerations, the best estimates were calculated from workers in the lowest exposure groups only. Additional estimates, based on the use of linear extrapolation of all dose groups, the absolute risk model, and U.S. EPA proposed methodology, were also calculated and ranged from 0.19 to 0.0014 (mg/kg-d)-1.

Reassessment of the cancer risks of benzene for California's drinking water program.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(4) not assignable

07-JUL-2005

(752)

**Remark:**

Hairy cell leukemia due to an occupational exposure to benzene?

Painter and spray/varnish painter was examd. who was periodically exposed over a period of 20 yr to concentrations of benzene in varnish paint and solvents.

The

patient was diagnosed as having contracted hairy cell leukemia at age 54. Hairy cell leukemia is a rare non-Hodgkin's lymphoma of low malignancy. According to the REAL-classification, it is considered to be a peripheral B-cell neoplasia. His physician had suspected evidence of an

occupational disease in the sense of Nr. 1303 of the German list of occupational diseases (diseases caused by the exposure to benzene, its homologues or styrene). Based upon a case history study and under the implementation of the current medical knowledge, the study discusses the existence

of a potential causal connection between an occupational exposure to benzene and the development of hairy cell leukemia. Following a evaluation of the patients medical data, the current status in medical knowledge cannot assume a causal connection of this kind.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(2) valid with restrictions

07-JUL-2005

(619)

**Remark:**

Benzene and multiple myeloma: appraisal of the scientific evidence

The purposes of this appraisal are to update, review and summarize epidemiological studies of the association of benzene with myeloma and to illustrate the use of the criteria developed by Sir A.B. Hill in evaluating of role of

benzene in the causation of multiple myeloma. Also reviewed are recent advances in the detection of specific chromosome lesions in myeloma cells and those associated with drug and chemical exposure. Epidemiological studies from the United States, Canada, United Kingdom, China, Italy, the Scandinavian countries, China and other countries were reviewed. Based on a thorough review and analysis of the existing scientific data according to well-established criteria, the authors concluded that: (1) There is strong evidence linking high levels of exposure to benzene with an increased risk of developing acute myelogenous leukemia. The evidence of this association satisfies all the Hill's criteria, and the relationship can be judged as causal in nature. Furthermore, cell-type specific analysis indicates that the threshold is most likely to be around 370 to 530 ppm-years. (2) In contrast, there is no scientific evidence to support a causal relationship between exposure to benzene or other petroleum products and the risk of developing multiple myeloma.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

07-JUL-2005

(97)

**Remark:**

Review of leukemia cell-type specific risks in epidemiologic studies of benzene workers. The purpose of this document was to review the human epidemiologic studies on benzene with respect to leukemia cell types. While benzene is clearly linked to leukemia in humans, the question of whether this effect is cell type specific is important. First, if benzene were causally linked to all leukemia cell types, the burden of benzene over-exposure would be larger. Secondly, in the quest to find a mechanistic explanation for benzene-induced leukemia, current views are that the cell type would be important. Five studies were selected from a set of 16 studies because they satisfied the two attributes that: (1) benzene exposure was measured quantitatively and (2) leukemia cell types were specified. These two features allow a formal assessment of dose response for benzene and leukemic cell types, an essential element for determining causality. The studies of Crump, 1994; Bond et al., 1986; Hayes et al., 1997; Rushton and Romaniuk, 1995; Ireland, 1997 were reviewed. General findings and conclusions of this review include the following. There is sufficient evidence to conclude that acute non-lymphocytic leukemia (ANLL) shows a dose response relationship with benzene. All five of the studies that are reviewed here provide either strong evidence or at least some indication of a possible relationship. To date, there

does not appear to be a relationship with other leukemia cell types and benzene. To improve the confidence that no other leukemia cell type is related to benzene, future assessments should strive to obtain larger sample sizes, and provide analyses of specific sub-types, rather than all non-ANLL leukemias. To date, there does not seem to be sufficient evidence to conclude that other LH cancers are related to benzene. The one study that does suggest a non-Hodgkin's leukemia (NHL) risk reports that it is strongest in the chemical industry, where associated exposures may be confounding variables. While benzene may be involved in conjunction with other agents, this one study does not seem to provide a strong enough rationale for suggesting that benzene experienced in more typical exposure scenarios can cause NHL. However, future, larger studies should be conducted to assess this further.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(1) valid without restriction

07-JUL-2005

(1019)

**Remark:**

A potential mechanism underlying the increased susceptibility of individuals with a polymorphism in NAD(P)H:quinone oxidoreductase 1 (NQO1) to benzene toxicity.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a two-electron reductase that detoxifies quinones derived from the oxidation of phenolic metabolites of benzene. A polymorphism in NQO1, a C609T substitution, has been identified, and individuals homozygous for this change (T/T)

have no detectable NQO1. Exposed workers with a T/T genotype have an increased risk of benzene hematotoxicity. This finding suggests NQO1 is protective against benzene toxicity, which is difficult to reconcile with the lack of detectable NQO1 in human bone marrow. The human promyeloblastic cell line, KG-1a, was used to investigate the ability of the benzene metabolite hydroquinone (HQ) to induce NQO1. A concentration-dependent induction of NQO1 protein and activity was observed in KG-1a cells cultured with HQ. Multiple detoxification systems, including NQO1 and glutathione protect against benzene metabolite-induced toxicity. Indeed, exposure to a noncytotoxic concentration of HQ induced both NQO1 and soluble thiols and protected against HQ-induced apoptosis. NQO1 protein and activity increased in wild-type human bone marrow cells (C/C) exposed

to HQ, whereas no NQO1 was induced by HQ in bone marrow cells with the T/T genotype. Intermediate induction of NQO1 by HQ was observed in heterozygous bone marrow cells (C/T). NQO1 also was induced by HQ in wild-type (C/C) human bone marrow CD34(+) progenitor cells. This data suggest that failure to induce functional NQO1 may contribute to the

increased risk of benzene poisoning in individuals homozygous for the NQO1 C609T substitution (T/T).  
**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005 (792)

**Remark:** Benzene, NAD(P)H:quinone oxidoreductase 1 (NQO1) and genetic susceptibility to cancer  
This review highlights recent developments in benzene toxicity and NAD(P)H:quinone oxidoreductase 1 (NQO1) polymorphism, and the subsequent risk of benzene-induced leukemia in humans. This was a commentary by M.T. Smith on the paper of Moran et al., (1999) in the same issue of the Proc Natl Acad Sci. on the potential mechanism of increased susceptibility of individuals to benzene toxicity with a polymorphism in NQO1. Benzene is metabolized in the liver to phenol, hydroquinone, catechol, which then travel to the bone marrow and can be activated by peroxidases to highly toxic quinones. NQO1 is capable of maintaining these quinones in their reduced form, thereby detoxifying them. NQO1 has been hypothesized to protect against benzene toxicity and individuals who lack NQO1 appear to be at higher risk of benzene poisoning. The lack of NQO1 activity in humans is the result of homozygous inheritance of two mutant alleles at position 609 in the NQO1 gene. Genotype-phenotype studies have confirmed that the homozygous 609 C -- > T change results in a lack of NQO1 activity and protein. Analysis of DNA isolated from subjects in Shanghai revealed that individuals who are homozygous for the C609T polymorphism were significantly more likely to be poisoned by benzene and were at elevated risk of contracting benzene-induced leukemia (Rothman et al., 1997). There appears to be a high incidence of mutant NQO1 allele in the Chinese population with approximately 20% of the population being homozygous mutants. These findings have generated considerable research interest in understanding NQO1 polymorphism and the mechanism(s) underlying the susceptibility of individuals to benzene toxicity. The review also provides a perspective on historical milestones as well as recent findings, and discusses current directions of research efforts.  
**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction  
07-JUL-2005 (1053)

**Remark:** NAD(P)H:quinone oxidoreductase (NQO1) converts benzene-derived quinones to less toxic hydroquinones and has been implicated in benzene-associated hematotoxicity. A point mutation in codon 187 (Pro to Ser) results in complete loss of enzyme activity in homozygous subjects, whereas those with 2 wild-type alleles have normal activity. The

frequency of homozygosity for the mutant allele among Caucasians and African Americans is 4% to 5% but is higher in Hispanics and Asians. Using an unambiguous polymerase chain reaction (PCR) method, we assayed nonmalignant lymphoblastoid cell lines derived from 104 patients with myeloid leukemias; 56 had therapy-related acute myeloid leukemia (t-AML), 30 had a primary myelodysplastic syndrome (MDS), 9 had AML de novo, and 9 had chronic myelogenous leukemia (CML). All patients had their leukemia cells karyotyped. Eleven percent of the t-AML patients were homozygous and 41% were heterozygous for the NQO1 polymorphism; these proportions were significantly higher than those expected in a population of the same ethnic mix ( $P = .036$ ). Of the 45 leukemia patients who had clonal abnormalities of chromosomes 5 and/or 7, 7 (16%) were homozygous for the inactivating polymorphism, 17 (38%) were heterozygous, and 21 (47%) had 2 wild-type alleles for NQO1.

Thus, NQO1 mutations were significantly increased compared with the expected proportions: 5%, 34%, and 61%, respectively ( $P = .002$ ). An abnormal chromosome no. 5 or 7 was observed in 7 of 8 (88%) homozygotes, 17 of 45 (38%) heterozygotes, and 21 of 51 (41%) patients with 2 wild-type alleles. Among 33 patients with balanced translocations [14 involving bands 11q23 or 21q22, 10 with  $inv(16)$  or  $t(15;17)$ , and 9 with  $t(9;22)$ ], there were no homozygotes, 15 (45%) heterozygotes, and 18 (55%) with 2 wild-type alleles. Whereas fewer than 3 homozygotes were expected among the 56 t-AML patients, 6 were observed; 19 heterozygotes were expected, but 23 were observed. The gene frequency for the inactivating polymorphism (0.31) was increased approximately 1.4-fold among the 56 t-AML patients. This increase was observed within each of the following overlapping cohorts of t-AML patients: the 43 who had received an alkylating agent, the 27 who had received a topoisomerase II inhibitor, and the 37 who had received any radiotherapy. Thus, the frequency of an inactivating polymorphism in NQO1 appears to be increased in this cohort of myeloid leukemias, especially among those with t-AML or an abnormality of chromosomes 5 and/or 7. Homozygotes and heterozygotes (who are at risk for treatment-induced mutation or loss of the remaining wild-type allele in their hematopoietic stem cells) may be particularly vulnerable to leukemogenic changes induced by carcinogens.

Prevalence of the inactivating 609C-->T polymorphism in the NAD(P)H:quinone oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(666)

**Remark:**

Benzene is a ubiquitous occupational hematotoxin and leukemogen, but people vary in their response to this toxic agent. To evaluate the impact of interindividual variation in enzymes that activate (i.e., CYP2E1) and detoxify (i.e., NQO1) benzene and its metabolites, we carried out a case-control study in Shanghai, China, of occupational benzene poisoning (BP; i.e., hematotoxicity), which we show is itself strongly associated with subsequent development of acute nonlymphocytic leukemia and the related myelodysplastic syndromes (relative risk, 70.6; 95% confidence interval, 11.4-439.3). CYP2E1 and NQO1 genotypes were determined by PCR-RFLP, and CYP2E1 enzymatic activity was estimated by the fractional excretion of chlorzoxazone (fe(6-OH)) for 50 cases of BP and 50 controls. Subjects with both a rapid fe(6-OH) and two copies of the NQO1 609C-->T mutation had a 7.6-fold (95% confidence interval, 1.8-31.2) increased risk of BP compared to subjects with a slow fe(6-OH) who carried one or two wild-type NQO1 alleles. In contrast, the CYP2E1 PstI/RsaI polymorphism did not influence BP risk. This is the first report that provides evidence of human susceptibility to benzene-related disease.

Further evaluation of susceptibility for hematotoxicity and hematological malignancy among workers with a history of occupational exposure to benzene is warranted. Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609 C -->T mutation and rapid fractional excretion of chlorzoxazone

**Source:****Reliability:**

07-JUL-2005

EXXON Biomedical Sciences East Millstone, NJ

(1) valid without restriction

(965)

**Remark:**

Risk of cancer and exposure to gasoline vapors. Until the introduction of self-service around 1970, service station workers in the Nordic countries were exposed to gasoline vapors. Based on measurements reported in the literature, the 8-hour time-weighted average benzene exposure was estimated to be in the range of 0.5-1 mg/m<sup>3</sup>. We studied the cancer incidence in a cohort of 19,000 service station workers from Denmark, Norway, Sweden, and Finland. They were identified from the 1970 censuses and followed through 20 years, where 1,300 incident cancers were observed. National incidence rates were used for comparison. The incidence was not increased for leukemia [observed = 28, standardized incidence ratio (SIR) = 0.9, 95% confidence interval (CI) 0.6-1.3] not for acute myeloid leukemia (observed = 13, SIR = 1.3, 95% CI 0.7-2.1). The incidence was slightly elevated for kidney cancer observed = 57, SIR =

1.3, 95% CI 1.0-1.7) and for pharyngeal, laryngeal, and lung cancer. A 3.5-fold risk of nasal cancer was found (observed = 12, SIR = 3.5, 95% CI 1.8-6.1). This cohort exposed to gasoline vapors with benzene levels (estimated to be 0.5-1 mg/m<sup>3</sup>) showed no excess risk of leukemia or acute myeloid leukemia, a 30% elevated risk of kidney cancer, and a previously unnoticed risk of nasal cancer.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(2) valid with restrictions

07-JUL-2005

(714)

**Remark:**

OBJECTIVE: To determine whether occupational exposure of men to hydrocarbons has adverse effects on the quality of their semen. DESIGN: Comparative study. SETTING: The rubber industry in Mexico City. PATIENT(S): Forty-eight workers who were exposed to hydrocarbons for 2-24 years and 42 unexposed workers. INTERVENTION(S): None. MAIN OUTCOME MEASURE(S): Environmental hydrocarbon concentrations were determined by continuous air monitoring in all areas of the factory. Analyses of semen samples were performed in accordance with World Health Organization criteria. RESULT(S): Hydrocarbon concentrations were as follows: ethylbenzene, 220.7-234 mg/m<sup>3</sup>; benzene, 31.9-47.8 mg/m<sup>3</sup>; toluene, 189.7-212.5 mg/m<sup>3</sup>; and xylene, 47-56.4 mg/m<sup>3</sup>. The number of subjects with ejaculates that had normal characteristics was greater in the unexposed group (76%) than in the exposed group (17%). More abnormal characteristics were found in the semen of exposed workers than unexposed workers, including alterations in viscosity, liquefaction capacity, sperm count, sperm motility, and the proportion of sperm with normal morphology. Some abnormal characteristics correlated with the number of years of exposure to the hydrocarbons. CONCLUSION(S): Damage to the spermatogenic process resulting from hydrocarbon exposure was demonstrated by an increased rate of abnormalities in the semen of exposed workers compared with unexposed workers. This information may be useful for conducting future analyses of reproductive risks related to exposure to high concentrations of hydrocarbons.

Additional Comments: No information was given on the organic solvents or other chemicals used at the rubber industry factory. It is not known whether other hydrocarbons or organic compounds besides BTEX were present in the air and whether they would be confounding variables.

It is interesting to note that 62% of the exposed and non-exposed population sample were smokers. However, the investigators noted that there was no statistically significant differences found in the semen profiles when the

samples were taken from smokers or non-smokers in the two groups of workers.

Semen quality of workers occupationally exposed to hydrocarbons.

**Source:**

EXXON Biomedical Sciences East Millstone, NJ

**Reliability:**

(2) valid with restrictions

07-JUL-2005

(278)

07-JUL-2005

12-OCT-2000

12-OCT-2000

**Remark:**

Acute myeloid leukaemia and exposure to organic solvents: a case-control study.

The objective was to study the relation between exposure to organic solvents and the risk of developing acute myeloid leukaemia (AML) in Caucasians aged 18 years and more.

Ninety-eight cases of AML were diagnosed from September 1986

to March 1990 in Novi Sad, Yugoslavia, and in two London hospitals from September 1988 and May 1994 and from July 1992 and July 1994, respectively. Two controls were matched

to each case by hospital, year of admission, gender and 5-year age group. Information on solvent exposure was collected by interview. Odds ratios (ORs) were derived with

conditional logistic regression. The degree of solvent exposure was determined by three experts blind to the status

of the subject with good agreement between them (the K coefficient ranged between 0.52 and 0.86). The response rate

for cases was 80%. Exposure to solvents was associated with the increased risk of AML (OR: 2.52; 95% CI: 1.45-4.39; p = 0.001) and those with probable exposure to solvents were found to have an odds ratio of AML over three times greater than non-exposed. We also found an elevated OR for exposure to oils (OR: 1.56) but this was not statistically significant.

There is no clear pattern of increasing risks with increasing duration of employment but a significant risk was found for exposures of 10 years or less. An induction period of less than 10 years or more than 30 years

was associated with a significantly raised OR. There was a significant excess of machinery mechanics and fitters among the cases.

**Source:**

ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

07-JUL-2005

(671)

- Remark:** Benzene and lymphohematopoietic malignancies in China.  
No abstract given in paper.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (485)
- Remark:** Biomarkers in the molecular epidemiology of benzene-exposed workers.  
No abstract given in paper.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (1055)
- Remark:** Background: Automotive gasoline contains benzene, 1,3-butadiene, 1,2-dibromoethane and 1,2-dichloroethane, and the combustion products include certain polycyclic aromatic hydrocarbons, all of which have shown mammary gland carcinogenicity in long-term bioassays. It is the aim of this paper to investigate whether men occupationally exposed to gasoline and its combustion products have an elevated risk of breast cancer.
- Methods: A nationwide register based case control study on male breast cancer morbidity was established among members of a pension fund, compulsory for all employees. Employment histories were reconstructed for each of 230 cases and 12,880 control subjects based on computerized records. The odds ratios, adjusted for socioeconomic status, were estimated by conditional logistic regression analysis.
- Results: When a lag time of at least 10 years was included, the odds ratio for breast cancer among men with over three months of employment in trades with potential exposure to gasoline and combustion products was 2.5 (95% confidence interval: 1.3-4.5). Among men younger than 40 years at the time of first employment, the odds ratio was 5.4 (2.4-11.9).
- Conclusions: This study supports the hypothesis that occupational exposure to gasoline vapors and combustion products may play a role in the causation of male breast cancer. This hypothesis warrants further evaluation particularly in women.  
Elevated risk for male breast cancer after occupational exposure to gasoline and vehicular combustion products.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (476)

**Remark:** A public health concern regarding hazardous air pollutants (HAP) is their potential to cause cancer. It has been difficult to assess potential cancer risks from HAP due primarily to lack of ambient concn. data for the general population. The USEPA Cumulative Exposure Project modeled 1990 outdoor HAP concns. across the US, which were combined with inhalation unit risk ests., to est. the potential increase in excess cancer risk for individual carcinogenic HAP. These were summed to provide an est. of cancer risk from multiple HAP. The anal. estd. a median excess cancer risk of 18 lifetime cancer cases/100,000 people for all HAP concns. Approx. 75% of estd. cancer risk was attributable to exposure to polycyclic org. matter, 1,3-butadiene, formaldehyde, benzene, and Cr. Consideration of some specific uncertainties, including underestimation of ambient concns., combining upper 95% confidence bound potency ests., and changes to potency ests., found that cancer risk may be underestimated by 15% or over-estd. by 40-50%. Other unanalyzed uncertainties could make these under- or over-ests. larger. This anal. used 1990 ests. of concns. and can be used to track progress toward reducing cancer risk to the general population. Estimating cancer risk from outdoor concentrations of hazardous air pollutants in 1990.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (1279)

**Remark:** Exposure to benzene, occupational stress, and reduced birth weight.

Objectives: The association between birth weight and exposure to benzene, work stress, and other occupational and environmental hazards was investigated.

Methods: In a large petrochemical industry, 792 pregnant workers were enrolled and followed up through delivery between May 1996 and December 1998. Exposure to benzene and other solvents was assessed by an industrial hygienist based on each woman's job title and workplace information. Other occupational and environmental exposures and personal information, including perceived work stress, exposure to noise, physical exertion at work, and passive smoking, were obtained by an interview questionnaire. Univariate and multivariate regression models were used to examine the individual and combined associations of occupational and environmental exposures with birth weight, with adjustment for major confounders including gestational age.

Results: In the univariate model, birth weight was negatively associated with exposure to benzene (-58 g (95% confidence interval (95% CI), -115 to -2)) and with work stress (-84 g (95% CI, -158 to -10)). In the multivariate model, there was a significant interaction

between exposure to benzene and work stress relative to reduced birth weight, after adjustment for other environmental and occupational exposures and personal variables. Adjusted mean birth weight was 3445 g (95% CI 3401 to 3489) among those with neither exposure, 3430 g for those with exposure to benzene only, 3426 g for those with work stress only, and 3262 g (95% CI 3156 to 3369) for those with both exposures. In other words, there was 183 g (95% CI 65 to 301) reduction in birth weight among those with both exposure to benzene and work stress compared with those with neither exposure. Other work or environmental factors could not explain these findings.

Conclusions: Low level exposure to benzene and work stress interact to reduce birth weight in this population.

**Source:**  
07-JUL-2005

ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
(186)

**Remark:**

Genetic susceptibility to benzene and shortened gestation: evidence of gene-environment interaction. This study investigated whether the association between low level benzene exposure and shortened gestation is modified by two susceptibility genes, CYP1A1 and GSTT1. This report includes 542 (302 nonexposed, 240 benzene-exposed) nonsmoking and nondrinking mothers of singleton live births at Beijing Yanshan Petrochemical Corporation between June 1995 and June 1997. Epidemiologic and clinical data and blood samples were obtained from mothers. Multiple linear regression models were used to estimate the associations of benzene exposure and genetic susceptibility with gestational age, adjusting for maternal age, education, parity, stress, passive smoking, prepregnancy weight and height, and infant's sex. Without consideration of genotype, benzene exposure was associated with a decrease in mean gestational age of 0.29 (standard error (SE), 0.12) week. When stratified by the maternal CYP1A1 genotype, the estimated decrease was 0.54 (SE, 0.12) week for the AA group, which was significantly greater ( $p = 0.003$ ) than that for the Aa/aa group, which showed no decrease in gestational age. When both CYP1A1 and GSTT1 were considered, the greatest decrease was found among exposed mothers with the CYP1A1 AA-GSTT1 absent group (0.79 (SE, 0.25) week) and the CYP1A1 AA-GSTT1 present group (0.50 (SE, 0.22) week). Among the nonexposed, genetic susceptibility alone did not confer a significant adverse effect. This study provides evidence of gene-environment interaction and supports further assessment of the role of genetic susceptibility in the evaluation of reproductive toxins.

**Source:**  
07-JUL-2005

ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
(1231)

- Remark:** Issues for discussion: benzene-induced leukemia-human studies.  
No abstract given in paper.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (86)
- Remark:** No abstract given in paper.  
Petroleum worker studies and benzene risk assessment.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (1018)
- Remark:** Rapid determination of benzene metabolites phenol and p-cresol in the urine of petrol station workers by gas chromatography.  
The determination of phenol and p-cresol in urine has been suggested as a suitable biomarker for the monitoring of benzene exposure of pump workers employed in petrol stations. A method for efficiently extracting a wide variety of drugs from urine for toxicological analysis by gas chromatography is presented. The sensitivity of the method is sufficient to monitor normal levels of phenol and p-cresol after exposure to benzene vapors. The detection limit of the method is low enough to measure urinary phenol and p-cresol at a concentration of 0.1 mg/l. The overall recoveries of urinary phenol and p-cresol relative to the internal standard, DPA, ranged between 95 and 96.5%. Our gas chromatographic method is a rapid, precise and economical one suitable to measure the metabolite benzene also in routine monitoring of phenol and p-cresol.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (615)
- Remark:** No abstract given in paper.  
Recent findings and new initiatives for epidemiologic research on benzene.
- Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (1268)
- Remark:** Occupational exposure to respiratory irritants may effect respiratory function in workers exposed to ambient pollutants in workplace air. A total of 567 male and 135 female workers employed in 2 chem. plants in Zagreb, Croatia, were studied. Ambient air pollutant concns. were measured. The mean age of men was 37 yr and mean duration of employment was 12 yr; a majority of these workers were smokers. The mean age of the women was 37 yr with a mean duration of employment of 14 yr; only one-third of the women smoked. An unexposed group of 340 male and 110 female unexposed workers was also studied. Acute and chronic work related symptoms were recorded for all workers.  
Ventilatory

capacity was measured by recording max. expiratory flow-vol. (MEFV) curves. There were higher prevalences for all chronic respiratory symptoms in exposed vs. unexposed workers particularly among women, a majority of which were non-smokers. Occupational asthma was recorded in 3 (0.5%) men and 2 (1.5%) women workers. Logistic regression anal. indicated the presence of chronic respiratory symptoms among exposed workers was primarily assocd. with the amt. of smoking. In addn., there were high prevalences of acute symptoms during work shift. Among chem. workers, these were greatest for eye irritation (male: 43.9%; female: 51.9%), dryness of the throat (male: 43.4%; female: 57.0%) and throat irritation (male: 37.4%; female: 56.6%).

Ventilatory capacity data among chem. workers demonstrated most of the measured tests, particularly FVC and FEV1, were significantly decreased vs. predicted ( $P < 0.01$  or  $P < 0.05$ ) values. In particular, non-smoking women exhibited abnormal lung function. The effect of smoking among exposed workers was demonstrated on all ventilatory capacity tests by regression anal. for all measured respiratory parameters. Both length of exposure and age were correlated with lung function loss for FVC. Measured pollutant concns. were for the most part within acceptable std. limits. The data suggested that in this population of chem. workers exposed to low pollutant concns., respiratory symptoms were primarily assocd. with smoking. Respiratory findings in chemical workers exposed to low concentrations of organic and inorganic air pollutants.

**Source:**  
07-JUL-2005

(805)

**Remark:**

**Objective:** The aim of the study was to investigate the immunotoxicity of benzene exposure, to establish the correlation between the exposure biomarkers and some immunological parameters, and to assess the possible influence of confounding factors on the results of immunological assay applicable in routine medical surveillance of benzene-exposed workers.

**Methods:** Forty-nine female workers in the shoemaking industry who were exposed to solvent mixtures and 27 nonexposed controls were examined. Workers were exposed to benzene concentrations of up to 15 ppm, and to toluene of up to 50 ppm.

**Results:** Significant differences in the levels of benzene and toluene in blood and phenols in post-shift urine between the exposed and the control group confirmed solvent exposure. The number of B-lymphocytes ( $P = 0.01$ ) was

lower  
in the shoe workers than in the controls. Significant correlation was found between the level of immunoglobulin G and benzene in the work atmosphere, while confounding factors had no impact on immunological values.

Conclusion: According to these results, exposure to benzene concentration lower than 15 ppm can induce depression of the circulating B-lymphocyte level and therefore this fact could be used to develop a promising method for health surveillance of benzene-exposed workers. However, considerably more effort in the research on benzene immunotoxicity, especially in the search for suitable health surveillance methods, is still required. Study of some immunological parameters in workers occupationally exposed to benzene.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (125)

**Remark:** No abstract given in paper.  
The Health Effects Institute's biomarker research in China.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (934)

**Remark:** A hand-saving HPLC method to measure urinary phenylmercapturic acid (PMA) was developed which allows approx.35 PMA detns. per day. The method involves the conversion of pre-PMA to PMA by the addn. of sulfuric acid to a urine sample, extn. into an ether-methanol mixt., followed by condensation under a nitrogen stream. The condensate was introduced to a ODS-3 column in a HPLC system, and PMA in the column was eluted into a mobile phase of acetonitrile:methanol:perchloric acid:water. The elution of PMA was monitored at 205 nm. One detn. was completed in 40 min. The method was applied to the anal. of end-of-shift urine samples from 152 workers exposed up to 210 ppm benzene, 66 workers exposed to a mixt. of benzene (up to 116 ppm), and toluene + xylenes (up to 118 ppm), and 131 non-exposed controls of both sexes. A linear regression was established between time-weighted av. intensity of exposure to benzene and urinary PMA. From the regression, it was calcd. that the urinary PMA level will be approx.6.4 mg/L after an 8-h exposure to benzene at 100 ppm, and that PMA in urine accounted for approx. 0.1% of benzene absorbed. No effects of sex, age, and smoking habit of individuals were

detected, and the effect of co-exposure to toluene and xylenes at the levels comparable to that of benzene was essentially nil, which indicates an advantage of PMA as a benzene exposure marker over mono- to tri-phenolic metabolites or t,t-muconic acid.

Urinary phenylmercapturic acid as a marker of occupational exposure to benzene.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
07-JUL-2005 (559)

**Type of experience:** other: indoor, outdoor and personal exposure

**Method:** Subjects  
The study population comprised 50 non-smokers, including 30 outdoor workers. Indoor monitoring was conducted in the participant's bedrooms, outdoor monitoring at 100 urban background sites, distributed over the area where the volunteers lived and spent their working day.

Collection of exposure data  
Campaigns were conducted over the working week (Mon - Fri). All sampling was carried out by passive means (Radiello Perkin Elmer). Outside samplers were placed inside protective shelters 3m above ground level. Indoor monitoring

were attached to ad hoc supports 1m above floor level in the

volunteers bedroom. Personal samplers were attached within the breathing zone and left open in the bedroom during the night. Analysis was by automatic thermal desorption GC.

**Remark:** This article describes the French contribution to the MACBETH programme (Monitoring Ambient Air Concentrations of Benzene in European Towns and Homes).

**Result:** Outdoor concentrations  
A mean outdoor concentration of 4.0 ug/m3 was obtained, based on 600 measurements (range 2.7 - 5.7 ug/m3, excluding all values >90th percentile).

Indoor concentrations  
A mean indoor concentration of 6.0 ug/m3 was obtained (range 4.5 - 8.3 ug/m3, excluding all values >90th percentile).

Personal exposure measurements  
Mean personal exposures were 10.3 ug/m3 (range 8.3 - 13.0 ug/m3, excluding 90th percentile). There was very little difference in exposure over the six campaigns between volunteers who worked indoors (range 7.9 - 15.3 ug/m3) and those who worked outside (range 8.1 - 11.3 ug/m3).

Total calculated personal exposure  
The total calculated personal exposure to benzene (based on the outdoor, indoor and personal exposure measurements collected during the study) was in a range 622 - 772 ug/hr/m3. This was lower than the measured personal exposure (1127 - 1759 ug/he/m3).

Temporal variations in benzene concentrations  
During the rush-hour (15.3 ug/m3), concentrations were approx. double those measured for the working week (mean 7.5 ug/m3).

**Source:** A.K. Mallett Surrey

**Conclusion:** The results show that outdoor concentrations of benzene were significantly lower than indoor levels, and that calculated estimates of personal exposure (derived by combining indoor and outdoor measurements) under-estimated actual personal exposure.

15-MAR-2002

(429)

**Type of experience:** other: concentrations in urban domestic and public microenvironments

**Method:** Selection of microenvironments  
Exposure to benzene (and other VOCs) was determined in a range of urban microenvironments (see results).

Sample collection and analysis  
Sampling was carried out between November 1999 and February 2000. The air samples were adsorbed onto Tenax/Carbotrap, then analysed using GC-MS. The LoD for benzene was 0.26 ug/m3.

**Result:** Mean concentration of benzene (ug/m3) in different microenvironments

homes 13.9 (n=64)  
offices 5.9 (n=12)  
restaurants 22.7 (n=6)  
bars 31.7 (n=)6  
department stores 10.5 (n=8)  
cinemas 15.5 (n=6)  
perfume shop 6.8 (n=3)  
libraries 8.8 (n=6)  
laboratories 4.2 (n=8)  
train stations 46.5 (n=12)  
coach stations 20.0 (n=12)  
roadside 49.6 (n=12)  
inside cars 203.7 (n=35)  
inside trains 24.3 (n=35.8)  
inside buses 20.2 (n=18)

Mean concentrations at heavily trafficked roadsides were exceeded by those in automobiles, and were comparable to those in bars and train stations. Other analyses showed that mean levels inside homes were greater than levels outdoors.

**Source:** A.K. Mallett Surrey

17-MAR-2002

(618)

**Type of experience:** other: biological monitoring (urinary S-phenylmercapturic acid)

**Remark:** BACKGROUND  
Oxidation of benzene to benzene oxide and subsequent conjugation with glutathione yields a pre-mercapturic acid which is further metabolised in the kidney and eliminated as the water soluble S-phenylmercapturic acid (S-PMA). This can be quantified using a competitive enzyme-linked immunosorbant assay (ELISA) with a working range of 40-1200 nmol/l S-PMA in urine.

#### METHOD VALIDATION

Results obtained using the ELISA method were compared with those using GC-MS. A correlation coefficient of 0.92 was obtained for thirty urine samples containing S-PMA within the working range of the ELISA. Potentially cross-reacting (hippuric acid, phenyl, benzylmercapturic acid) added to urine did not interfere with the ELISA method.

#### METHOD APPLICATION

Random post-shift urine samples were collected from 32 non-smoking individuals with occupational exposure to benzene (17 male, 15 female; petrochemical refineries, coke ovens, chemical manufacturing plants, clean-up operations in Europe and North America). Samples were analysed for S-PMA and creatinine and results compared with data from a control population of non-occupationally-exposed subjects. The mean analysed level of S-PMA in urine for the controls was 0.9 umol PMA/mol creatinine (range 0-1.9) while values for the occupationally-exposed group were between 0 and >90 umol S-PMA/mol creatinine.

**Source:** A.K. Mallett Surrey

**Conclusion:** The authors suggest the method may be used for routine biological monitoring of workers occupationally-exposed to benzene.

09-SEP-2002

(49)

**Type of experience:** other: air monitoring

**Method:** SAMPLE COLLECTION AND ANALYSIS  
Diffusive- and active air sampling was undertaken at 9 locations in Paisley (covering urban centre, urban background and kerbside environments; October 1996-July 1998) and at 5 locations outside Paisley (urban background and kerbside environments; April 1997-July 1998). Active sampling used tubes containing Anasorb CMS and an activated molecular sieve, arranged in series and connected to a personal sampling pump. Diffusive sampling employed tubes containing heat-activated Perkin-Elmer C106 which were fixed to a solid object (eg lamppost) 2-3 m above the ground. Active samples were analysed using GC-FID or GC-MS,

diffusive samples were analysed using automated thermal desorption interfaced with GC-MS.

#### METEROLOGICAL DATA

Meteorological information was obtained from two local, automatic weather stations.

#### Result:

METEROLOGICAL DATA (average values)

Air temperature: 10.6 degrees C

Rainfall: 2.7 mm

Wind speed: 2.0 m/s

Solar energy: 184 W/m<sup>2</sup>

Humidity: 74%

Pressure: 1004 mbar

Wind direction: 172 degrees

#### ACTIVE MONITORING FOR BENZENE

Atmospheric benzene levels were below the limit of detection

(1.9 ppb) for 12 of the 14 sites. Mean benzene levels of 1.6

ppb (max 1.68 ppb) were detected in the urban centre of Paisley, with 4.4-7.5 ppb (max 16.8 ppb) detected at an urban centre kerbside in Paisley. The urban centre kerbside value decreased to an undetectable level (ie <1.9 ppb) following pedestrianisation of this area.

#### DIFFUSIVE MONITORING FOR BENZENE

Benzene was present in all analysed samples. Generally, kerbside samples in central sites gave rise to higher readings (mean range 1.28-1.55 ppb) than non-kerbside samples (0.41-0.87 ppb). There was a trend to decreasing concentrations during summer periods (possible reflecting thermal and photo-oxidative removal of benzene).

#### Source:

A.K. Mallett Surrey

#### Conclusion:

Vehicle movements had a major influence on the concentration of benzene in air. Peak monthly averages were of the order 5-6 ppb, with minima of <0.5 ppb. Traffic intervention programmes (i.e. pedestrianisation) influenced atmospheric concentrations over a wide area.

16-OCT-2002

(800)

#### Type of experience:

other: biological monitoring (urinary S-phenylmercapturic acid)

#### Method:

##### SAMPLE COLLECTION

Pre- and post-shift urine samples were collected from workers from the petroleum- and petrochemicals sector, combustion-process workers from the steel industry and workers involved in decontamination of land subject to a spill of chemical solvents. Reference samples were non-smoking controls with no exposure to chemicals.

##### URINARY PMA MEASUREMENTS

Urine samples were incubated with a limiting concentration of anti-S-phenylmercapturic acid (anti-PMA) polyclonal antibody in a multi-well microtitre plate coated with

**Result:** PMA-bovine serum albumin conjugate. The bound PMA fraction was subsequently incubated with an alkaline phosphatase-linked anti-sheep antibody, followed by addition of p-nitrophenyl phosphate (substrate). The absorbance of the released p-nitrophenol was determined at 405 nm. A series of standards (PMA in urine) were processed in parallel. Results were standardised against urinary creatinine (quantified using a commercially-available kit). Background levels of urinary PMA for control subjects (n=32) were in a range 0 to 1.9 umol/mol creatinine (median 0.9 umol/mol). There was no difference in pre- and post-shift values for the controls. (Limit of detection 40 nmol/l urine; equivalent per mol creatinine not stated).

Analysis of pre-shift urine samples for a group of refinery workers (n=19) returned values in a range 0.5-2.4 umol PMA/mol creatinine (median 1.1 umol/mol), while post-shift values (n=14) were in a range 0.6-89.3 umol/mol (median 1.6 umol/mol). In contrast, post -shift values of 0.6-3.0 umol/mol creatinine were found in another group of refinery workers (n=16; median 1.2 umol/mol; no pre-shift values). The authors note that the highest level of urinary PMA found in these subjects (89.3 umol/mol) is equivalent to an 8-hr TWA exposure of approx. 4 ppm benzene.

Post-shift urinary PMA levels for two groups of workers exposed to benzene during combustion process manufacture of steel were in a range 0.7-62.5 umol/mol creatinine (n=39; median 1.4 umol/mol) and 0.7-56.7 umol/mol (n=41; median 2.7 umol/mol). (No pre-shift data presented.)

**Source:** PMA was also detectable in post-shift urine from workers involved in clean-up of a chemicals spill (n=41; range = 0.5-32.7 umol/mol creatinine; median 4.2 umol/mol).  
**Conclusion:** A.K. Mallett Surrey  
This enzyme-linked immunosorbent assay demonstrated elevated levels of S-phenylmercapturic acid in urine from 5 groups of non-smoking workers compared to that present in control subjects. The authors suggest that the method may be used for rapid, high-throughput analysis of samples from individuals with suspected exposure to benzene.

25-JAN-2003

(899)

**Type of experience:** other: benzene exposure from petroleum products

**Remark:** This reference presents a summary of benzene levels in gasoline and crude oil; the authors suggest that this information may be helpful when performing retrospective exposure assessments in epidemiological studies. Published studies indexed by bibliographic databases (NIOSH-TIC, CISILO, SCI), and technical reports from API or CONCAWE

formed the basis of this compilation.

The concentration of benzene in crude oil is relatively low, with mean values of 0.16-0.33% w/w reported.

A mean concentration of 1 to 4% benzene is historically prevalent in European gasoline, regulatory controls introduced in January 2000 limit this to a maximum of 1%.

Mean historical values in the USA are generally lower than those in Europe at around 2%, while the current reformulated gasoline programme specifies that levels should not exceed 1%.

The report includes extensive tabulated data reporting mean, maximum and minimum benzene concentrations in gasoline streams obtained and analysed in various parts of the world.

**Source:** A.K. Mallett Surrey  
25-JAN-2003

(1212)

**Type of experience:** other: parent and child exposure assessment

**Method:**

## SUBJECTS AND METHODS

Participants were recruited from three public day-care centres in Rouen, France, and comprised 42 members from 20 families (22 non-smoking parents, 21 children age 2-3 yr old).

Indoor benzene exposure measurements (passive monitoring, axial diffusive sampling) were made in parental and child bedrooms and in the day care centres. Background levels outside the day care centre were also quantified. Each parent was also fitted with a personal sampler in the breathing zone during the day; this was left open in the bedroom during the night. Measurements were performed Monday morning-Friday evening during October-December 1999.

Two urine samples were also collected from the parents and children (morning and evening) and stored frozen (-20 degrees C).

The children could not be fitted with personal monitoring equipment, hence benzene exposure was calculated based upon the time-weighted average of the home/bedroom (15 hr), day-care centre (8 hr) and outdoor (1 hr) benzene concentration for each child.

Personal benzene exposure for adults and children was calculated using physiological constants derived by US-EPA (Exposure Factors Handbook (1997) EPA/600/P-95/002Fa).

## ANALYTICAL METHODS

Analysis of personal samples used automatic thermal desorption followed by GC-FID. Creatinine in urine was measured spectrophotometrically using a commercial test kit.

The concentration of muconic acid in urine was quantified after concentration (Bond Elut extraction) followed by HPLC with UV detection. Urinary hydroquinone was quantified (after hydrolysis) by HPLC with fluorometric detection. Metabolite concentrations were expressed by gram creatinine.

(Values below the limit of detection were set at one-half of the LoD; LoD not reported.)

#### STATISTICAL METHODS

Results were described using descriptive statistics. Differences between groups (parents versus children) were analysed using paired t-tests. Data were transformed to natural logarithms prior to analysis.

#### Result:

##### PERSONAL BENZENE EXPOSURE AND UPTAKE

There was no significant difference between bedroom concentrations for parents and children, however personal exposure for parents was significantly greater than for children:

	Parent	Child
Bedroom concentration:	9.2	10.9 ug/m <sup>3</sup> (n/s)
Personal exposure:	14.4	11.1 ug/m <sup>3</sup> (P<0.04)

Mean indoor benzene concentrations within the day care centres were in a range 7.9-35.5 ug/m<sup>3</sup>, while the corresponding mean outdoor levels were 3.5-7.6 ug/m<sup>3</sup>.

Based on these values, the calculated uptake of benzene was significantly greater for children (5.7 ug/kg bw/d) than for parents (2.5 ug/kg bw/d; P<0.0001).

##### URINARY MUCONIC ACID AND HYDROQUINONE

Urinary MA concentrations were in the range 0.01-13.47 mg/g creatinine for children and 0.004-9.04 mg/g creatinine for parents (samples collected over 5 days). Urinary HQ was 0.14-24.75 mg/g creatinine and 0.01-21.72 mg/g creatinine for children and parents during the same period.

Mean MA concentration in adult urine (0.73 mg/g creatinine) was not significantly different from the mean concentration in children's urine (0.85 mg/g creatinine), whereas mean HQ was significantly higher (P<0.0001) in children (2.51 mg/g creatinine) than in parents (1.69 mg/g creatinine).

There was no statistically significant correlation between mean benzene exposure and urinary metabolite levels.

Comment: The authors note that ingestion of sorbic-acid based preservatives or the presence of dietary phenolics may have confounded any relationship between urinary MA and HQ

and external benzene exposures in this study. They also note that creatinine elimination increases with body mass, and that excretion of MA and HQ standardised per g creatinine approximates excretion standardised per kg body weight thereby providing a surrogate estimate of internal exposure.

Based on this analysis, they suggest that children have a higher internal exposure than adults. The basis of this conclusion is not clear, however, given the probable contribution of dietary precursors to urinary MA and HQ. A.K. Mallett Surrey

**Source:****Conclusion:**

Muconic acid and hydroquinone do not appear to be reliable, specific markers of low benzene exposure due to potential confounding by food-borne precursors of these substances. This may explain the absence of correlation between urinary metabolite levels and benzene exposure in this study.

16-MAR-2004

(638)

**Type of experience:** other: benzene oxide-albumin adducts in human blood**Method:**

An expanded version of this study and its methods was reported subsequently by Qu et al. (2003) Validation and evaluation of biomarkers in workers exposed to benzene in China. Research Report 115, Health Effects Institute, Boston MA.

## SUBJECTS AND METHODS

Thirty benzene-exposed workers were recruited from three factories in Shanghai, China where benzene was used as a rubber solvent, in the manufacture of adhesive tape or in paint. Forty three control workers (frequency matched with exposed group by gender and age) were recruited from a sewing machine factory and an administrative facility in the same geographic region.

## EXPOSURE MEASUREMENTS

Exposure to benzene was assessed measured by personal monitoring (passive; full work shift on 5 or 6 consecutive days).

## ADDUCT MEASUREMENTS

Benzene oxide-albumin adducts (BO-Alb) in blood were determined by GC-MS in negative chemical ionisation mode. (Blood collection details not given).

## STATISTICAL METHODS

Results of analyses for BO-Alb in blood and personal exposure data for the exposed worker population were natural log transformed and subject to regression analysis. This analysis was performed twice, once on the raw BO-Alb results and again after subtraction of the geometric mean value for the controls from data for the exposed population. Within

**Result:**

and between subject components were determined by one way ANOVA of logged exposure data. Exposure monitoring returned an overall geometric mean of 11.0 ppm benzene for exposed workers (range 0.2-302 ppm). Measurements in control workers revealed that only 3 of 30 measurements were above the detection limit of 0.016 ppm (measured values: 0.047, 0.052, 0.110 ppm).

Levels of BO-Alb adducts in exposed workers (GM = 378 pmol/g) were greater than those in controls (GM = 115 pmol/g protein).

Graphical results show that a log-log plot of adduct concentration versus exposure concentration was linear. After correction for background adduct levels, the following relationship (n=26, R<sup>2</sup> = 0.359, p = 0.0012) was obtained:

$$\text{BO-Alb} = 4.38 + 0.497 \times (\ln[\text{benzene, ppm}])$$

Since the estimated slope of 0.497 was less than one log, the authors infer that the rate of BO-Alb production decreased or levelled-off at higher benzene exposures.

The initial linear rate of BO-Alb production (at low benzene exposures) was estimated as 39.5 pmol BO-Alb/g protein/ ppm benzene. The rate of adduct production became non-linear at exposures of approx. 10 ppm and above. Comment: Since benzene is a substrate for CYP2E1, the authors suggest these findings may be consistent with saturation of benzene metabolism.

**Source:**

A.K. Mallett Surrey

**Conclusion:**

The concentration of benzene oxide-albumin adducts in blood was greater in benzene-exposed workers relative to unexposed workers. Dose-response data indicated that the increase was less than proportional to personal benzene exposure over the range 0.2-302 ppm, with a deviation from linearity occurring at around 10 ppm.

31-MAR-2004

(915) (923)

**Type of experience:** other: Pliofilm exposure update**Remark:**

Past estimates of benzene exposure for the Pliofilm cohort have been criticised for potentially under- and/or over-predicting workplace levels, thereby introducing uncertainty into cancer risk estimates (i.e. over-predicting risk at low-levels if exposure estimates are too high and vice versa). This analysis uses Monte Carlo techniques (that is, probability distributions, rather than single mean or maximum values) to estimate the likely distribution of

benzene exposures for various job categories at the three Pliofilm facilities (Akron I, Akron II, St Mary's).

All estimates of equivalent airborne benzene concentration were characterised by a probability distribution to account for the possible variability and/or uncertainty in the underlying data. The probabilistic exposure modelling was developed using Crystal Ball software (Decisioneering Inc, Denver, CO) and run for 10,000 iterations.

Airborne exposures for the different job categories utilised background and peak benzene air concentrations and their relative exposure durations (supporting data included as an appendix in the publication). Historic hygiene data, collected during 112 surveys at the three sites, where benzene concentrations were less than the TLV of 25 ppm (in place 1963-1974) or 10 ppm (1975-1976) were assumed to represent background levels in air. Similarly, surveys that included values above the appropriate historic TLV were assumed to represent peak exposure values. Exposure duration, respirator use and efficiency, extent of dermal exposure and length of work week (including allowances for overtime) were among factors possibly modifying the exposure estimates.

Results are presented as 8 hr TWA equivalent airborne concentrations, incorporating uptake via both inhalation and skin contact, with adjustments for fractional uptake, respirator efficiency, length of workweek etc.

Estimated equivalent airborne benzene concentrations at the St Mary's site were highest for four job categories (Neutraliser, Quencher, Knifeman, Spreader) and typically ranged from 50-90 ppm at the 50th percentile during 1939-1946 (lower during 1942-1945), and 10-40 ppm during 1947-1976. These estimates were 2-3 fold greater than for other jobs in the Pliofilm process, and about 1.5 fold less than those estimated at the 95th percentile. Equivalent exposure estimates at the Akron I and II sites were 1.5 fold greater than for St Mary's, however there was less confidence in the values due to a lack of hygiene data for these facilities.

The authors suggest that these new exposure estimates incorporate what is considered to be the most likely range of plausible exposure values and provide a better characterisation of the potential workplace exposure for the Pliofilm cohort.

**Source:**  
26-APR-2004

A.K. Mallett Surrey

(1257)

**Remark:** Chromosomenuntersuchungen bei 10 gegenueber Benzol exponierten Beschaeftigten in der Benzol-Fabrikation zeigten 1-3 % Chromosomenaberrationen. Die Phenolausscheidung im Urin lag bei 31-65 mg/l.

**Source:** BASF AG Ludwigshafen

**Reliability:** (2) valid with restrictions  
acceptable study, meets basic scientific principles

24-NOV-1999 (388)

**Remark:** Von 1994-09/96 wurde ein Fall von Haut- und Augenreizung nach lokaler akzidentieller Einwirkung von Benzol beobachtet, der zur weiteren Behandlung in die Klinik eingewiesen wurde.

**Source:** BASF AG Ludwigshafen

**Reliability:** (2) valid with restrictions  
basic data given, acceptable restrictions

24-NOV-1999 (75)

**Remark:** A cluster of ten cases of Hodgkin's disease was identified within the active workforce of a large chemical manufacturing firm over a 23-year period. In the total workforce of 62000 person-years, an expected number of 2.01 cases was calculated, giving a standardized incidence ratio of 497 (95 % CI 238-915). A nested case-control study was undertaken with 200 controls. For 11 selected chemicals (incl. benzene) exposure odds ratios were calculated. No substance emerged as a likely candidate for explaining the observed Hodgkin's disease cluster.

**Source:** BASF AG Ludwigshafen

**Reliability:** (2) valid with restrictions  
acceptable study, meets basic scientific principles

24-NOV-1999 (1104)

**Remark:** Biomonitoring measurements were performed during repair operation in a ethylbenzene plant between 1993 and 1996. In the course of improvement of technical, organizational, and use of personal protective equipment urine levels of muconic acid were decreased.

**Source:** BASF AG Ludwigshafen

**Reliability:** (2) valid with restrictions  
acceptable study, meets basic scientific principles

24-NOV-1999 (123)

**Remark:** Study on biomonitoring in 43 workers exposed to low levels of benzene, ethylbenzene, and styrene. The concentration of benzene in air was <0.01 -0.13 ppm. Samples collected in 1999 are well below 1.7 mg muconic acid / g creatinine.

**Source:** BASF AG Ludwigshafen

**Reliability:** (2) valid with restrictions

11-FEB-2000                      acceptable study, meets basic scientific principles                      (272)

### 5.11 Additional Remarks

**Type:** other: A new dose model for assessment of health risk due to contaminants in air.

**Remark:** The problem of making quant. assessments of the risks assocd. with human exposure to toxic contaminants in the environment is a pressing one. This study demonstrates the capability of a new computational technique involving the use of fuzzy logic and neural networks to produce realistic risk assessments. The approach to this challenge tested here is to use a new model incorporating sophisticated artificial intelligence algorithms. Exposure assessment often requires that a no. of factors be evaluated, including exposure concns., intake rates, exposure times, and frequencies. These factors are incorporated into a system that can "learn" the relevant relationships based on a known data set. The results can then be applied to new data sets and thus be applied widely without the need for extensive measurements. In this anal., an example is developed for human health risk through inhalation exposure to benzene from vehicular emissions in the cities of Auckland and Christchurch, New Zealand. Risk factors considered were inhaled contaminant concn., age, body wt., and activity patterns of humans. Three major variables affecting the inhaled contaminant concn. were emissions (mainly from motor vehicles), meteorol. conditions (wind speed, temp., and atm. stability), and site factors (hilly, flat, etc.). The results are preliminary and used principally to demonstrate the technique, but they are very encouraging.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(918)

**Type:** other: New PBPK model applied to old occupational exposure to benzene.

**Remark:** An intensive program of benzene monitoring using new techniques was undertaken in Western Europe in the late 1960s and early 1970s. Significant exposure was found in the transport of benzene and gasoline, particularly during the loading of barges, and during the loading and operation of sea-going vessels. The ceiling threshold limit value of 25 ppm recommended at that time generated problems in assessing exposure, so alternative criteria were proposed. During that period some shore-based exposures were reported, and their significance was discussed in several articles. The information gained at that time is reexamined by physiologically based pharmacokinetic (PBPK) modeling and is used to help validate an improved PBPK model, which is described and tested on results from experimental exposure in a companion article. The old field data, comprising five specific studies, confirm the relevance of modeling to assessment of occupational exposure, and demonstrate its value for interpretation of field data, which is seldom as complete, systematic, or accurate as that obtained in experimental work. The model suggests that metabolism of

benzene in humans may not be restricted to the liver. Sites and processes of metabolism merit further investigation.

Additional Comments: PBPK modeling of benzene exposure predicts measurements of exhaled breath following occupational exposure with reasonable accuracy. However, prediction of concentration of phenol in urine is much less accurate. Further work is needed to confirm modeling systems for lower levels of exposure and for other biological monitoring indices, such as the currently indicators, S-phenylmercapturic acid or t-t-muconic acid in the urine. Further refinements in modeling may also improve accuracy. The PBPK model also has a skin absorption compartment that allows for the assessing possible contribution of dermal exposure to the total uptake.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

07-SEP-2000

(1034)

**Type:** other: Analysis of S-phenyl-L-cysteine in globin as a marker of benzene exposure.

**Remark:** An assay has been developed to det. S-phenylcysteine (SPC) in globin as a potential biomarker for exposure to benzene. The sensitivity of the assay is <20 pmol SPC g-1 globin. Following acidic hydrolysis of the protein, the modified amino acid is purified by reversed-phase cartridge chromatog. and HPLC, prior to conversion to the tert-butyldimethylsilyl deriv. and GC-MS selected ion recording. Quantitation is achieved using the internal std. [2H5]-SPC, and calibration lines were established using a synthetic peptide Leu-His-SPC-Asp-Lys. Control human globin was found to contain ca 30 pmol SPC g-1 globin in 2 populations. The source of the apparent background level of SPC is unknown.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(479)

**Type:** other: Structure and validation of a pharmacokinetic model for benzene.

**Remark:** A pharmacokinetic model for benzene has been developed and validated for the inhalation aspects of its operation. The validation shows reasonable agreement between the model outputs and human biological data for phenol in urine, benzene in alveolar air, and benzene in mixed exhaled air.

Additional Comments: Pharmacokinetic modeling is a useful method for simulating the absorption, distribution, metabolism and excretion of chemicals and their metabolites within living organisms. A physiologically based pharmacokinetic (PBPK) model has been developed that may be used to examine benzene pharmacokinetics in the human body. The PBPK model reported in this study is believed to be unique in two aspects. First, its structure has been

elaborated by the addition of a dermal entry module , which allows for the simultaneous modeling of benzene uptake by inhalation and dermal absorption. Second, it has been extensively validated for inhalation using data for phenol in urine and benzene in breath that was collected after exposing a human volunteer to benzene at known (calculated and measured) inhalation concentrations. There was reasonable agreement between the predicted amount of phenol (entire model output) and the validation data phenol rate (minus background). Those parts of the model outputs that could be compared with outputs for exhaled breath also compared favorably. The discrepancy between the model outputs and the validation data was more pronounced for benzene in exhaled breath than for phenol in urine. The pharmacokinetic model has limitations in two areas. The first limitation relates to its dermal uptake mechanisms and the uncertainty in flux rates, which cannot compensate for changing benzene concentrations within mixtures or to compensate for lag time during short exposure episodes. The second limitation centers around the metabolic process definitions used in the model. It calculates total metabolites (and phenol as a proportion) using a single Michaelis-Menten equation and this approach does not attempt to consider the multistage process of benzene metabolism.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

20-JUL-2000

(1036)

**Type:** other: Analysis of indoor concentrations of benzene using an air-quality model.

**Remark:** Indoor benzene concns. were measured in 26 residential houses in Kuwait, located in zones of different activity levels. Pumped (active) sampling was conducted using 12 sampling tubes over a 24-h period to measure indoor and outdoor concns. simultaneously. Time-av. indoor concns. varied linearly with time-av. outdoor concns. in accordance with a mass balance-based indoor air quality model in which source and sink terms were incorporated. Regression anal. detd. benzene adsorption rates, which appeared in the model removal and source terms. The removal rate parameter was 0.12-2.16/h; the source term parameter was 0.60-76.07 mg/h. Houses were then divided into 3 groups according to their benzene source strengths (<1.0 mg/h, 1-10 mg/h, 10-50 mg/h). Qual., these levels depended on the characteristics of occupants (e.g., smoking and gas cooker use, no. of cars, parking area) and location of the building.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(135)

**Type:** other: Use of a mathematical model of rodent in vitro benzene metabolism to predict human in vitro metabolism data.

**Remark:** Benzene, a ubiquitous environmental pollutant, is known to cause leukemia and aplastic anemia in humans and hematotoxicity and myelotoxicity in rodents. Toxicity is thought to be exerted through oxidative metabolites formed in the liver, primarily via pathways mediated by cytochrome P450 2E1 (CYP2E1). Phenol, hydroquinone and trans-trans-muconaldehyde have all been hypothesized to be involved in benzene-induced toxicity. Recent reports indicate that benzene oxide is produced in vitro and in vivo and may be sufficiently stable to reach the bone marrow. Our goal was to improve existing mathematical models of microsomal benzene metabolism by including time course data for benzene oxide, by obtaining better parameter estimates and by determining if enzymes other than CYP2E1 are involved. Microsomes from male B6C3F1 mice and F344 rats were incubated with [(14)C]benzene (14 microM), [(14)C]phenol (303 microM) and [(14)C]hydroquinone (8 microM). Benzene and phenol were also incubated with mouse microsomes in the presence of trans-dichloroethylene, a CYP2E1 inhibitor, and benzene was incubated with trichloropropene oxide, an epoxide hydrolase inhibitor. These experiments did not indicate significant contributions of enzymes other than CYP2E1. Mathematical model parameters were fitted to rodent data and the model was validated by predicting human data. Model simulations predicted the qualitative behavior of three human time course data sets and explained up to 81% of the total variation in data from incubations of benzene for 16 min with microsomes from nine human individuals. While model predictions did deviate systematically from the data for benzene oxide and trihydroxybenzene, overall model performance in predicting the human data was good. The model should be useful in quantifying human risk due to benzene exposure and explicitly accounts for interindividual variation in CYP2E1 activity.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

20-JUL-2000

(702)

**Type:** other: Assessing the health risks of benzene: a report on the benzene state-of-the-science workshop.

**Remark:** No abstract given in the paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(642)

**Type:** other: Physiological modeling of the toxicokinetic interactions in a quaternary mixture of aromatic hydrocarbons.

**Remark:** The available data on binary interactions are yet to be considered within the context of mixture risk assessments because of our inability to predict the effect of a third or fourth chemical in the mixture on the interacting binary pairs. Physiologically based toxicokinetic (PBTK) models represent a framework that can be potentially used for predicting the impact of multiple interactions on component kinetics at any level of complexity. The objective of this study was to develop and validate an interaction-based PBTK model for simulating the toxicokinetics of the components of a quaternary mixture of aromatic hydrocarbons [benzene (B), toluene (T), ethylbenzene (E), m-xylene (X)] in the rat. The methodology consisted of: (1) obtaining and refining the validated individual chemical PBTK models from the literature, (2) interconnecting all individual chemical PBTK models at the level of liver on the basis of the mechanism of binary chemical interactions (e.g., competitive, noncompetitive, or uncompetitive metabolic inhibition), and (3) comparing the a priori predictions of the interaction-based model to corresponding experimental data on venous blood concentrations of B, T, E, and X during mixture exposures. The analysis of blood kinetics data from inhalation exposures (4 h, 50-200 ppm each) of rats to all binary combinations of B, T, E, and X was suggestive of competitive metabolic inhibition as the plausible interaction mechanism. The metabolic inhibition constant ( $K(i)$ ) for each binary combination was quantified and incorporated within the mixture PBTK model. The binary interaction-based PBTK model predicted adequately the inhalation toxicokinetics of all four components in rats following exposure to mixtures of BTEX (50 ppm each of B, T, E, and X, 4 h; 100 ppm each of B, T, E and X, 4 h; 100 ppm B + 50 ppm each of T, E, and X, 4 h). The results of the present study suggest that data on interactions at the binary level alone are required and sufficient for predicting the kinetics of components in complex mixtures.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(463)

**Type:** other: Benzene induced genotoxicity: a different perspective.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(316)

**Type:** other: Review of potential reproductive and developmental effects of benzene.

**Remark:** In this review by Irvine, relevant animal studies were examined to assess the potential reproductive effects of inhaled benzene. There are species differences between rats and mice in sensitivity with regard to potential reproductive effects of benzene exposure. Rats appear less sensitive with effects on testis weight only manifest at 6600 ppm after approximately 13 weeks exposure (Wolf, et al., *AMA Arch. Ind. Health*, 14: 387-398, 1956). Female rats were not assessed at this level. An exposure level of 300 ppm in mice over 13 weeks elicited histopathological changes to male and female reproductive organs (Ward et al., 1982 in "Toxicology of Petroleum Hydrocarbons", eds. MacFarland, HN et al., pp. 26-45, Princeton Scientific Publishing). The difference between rats and mice may be a function of differences in respiration rates rather than selective toxicity. For both mice and rats, other benzene toxicities were observed at these exposure levels, indicating that reproductive effects are not primary manifestations of benzene toxicity.

Male guinea pigs showed slight effects on testes weight at 88 ppm over about 9.5 months, but not with a shorter (4 week) dosing period, and rabbits showed pathological changes in the testes (degeneration of seminiferous tubules) at 80 ppm over approximately 8 months (Wolf, et al., 1956). However, the confidence in the findings in guinea pigs and rabbits is low because the group sizes were very small and no quantitative or comparative data were available.

Although changes to male reproductive organs at high exposure levels have been identified, there appears to be no functional assessments of the potential effects of benzene inhalation on male fertility. There is, however, an acceptable study of female fertility (Kuna et al., *Amer. Coll. Toxicol.*, 11: 275-282, 1992), which demonstrates a lack of effect on fertility and fecundity in the rat at exposure levels up to 300 ppm. Although fertility was unaffected, there was evidence of developmental toxicity, manifest as effects on weaned pup body weight and liver weight at 300 ppm, and a trend for lower pup weights (although not statistically significant) at 30 ppm. It has been reported that benzene-induced carcinogenesis in rats is increased if exposures are begun during embryonic development (Gestation Day [GD] 12) compared to postnatal exposures at 13 weeks (Maltoni et al., *Environ. Health Perspect.*, 82: 109-124, 1989). This is important information, particularly since there appears to be no studies investigating the potential reproductive effects of benzene in in utero exposed progeny.

In assessing the potential developmental effects of inhaled benzene, nine relevant animal studies were reviewed. General conclusions of this review indicate that fetotoxicity (fetal weight and skeletal ossification retardation) has been a

consistent observation in most benzene inhalation developmental toxicity studies conducted. At high exposure levels, this has been associated with maternal toxicity but, with one exception, it has been observed at lower levels in the absence of maternal changes (although few studies check the dams at appropriate timepoints for effects on the most sensitive parameter of benzene toxicity- that of hematological changes). This indicates that the fetus may be slightly more sensitive to benzene toxicity than the adult. However, despite the observed fetotoxicity, there have been no consistent findings of embryoletality or teratogenicity associated with benzene treatment. No studies have shown an increase in permanent structural fetal abnormalities.

The rat studies have established a NOAEL for developmental toxicity of approximately 40 ppm for benzene inhalation exposure. NOAELs have not been established for the mouse and rabbit, but the LOAELs from conventional studies, where only mild fetotoxicity was observed, are 500 ppm. If the hemopoietic studies of Keller and Snyder, (1986, 1988) are considered relevant for developmental toxicity, then the NOAEL for the mouse is exceptionally low (<20 ppm).

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(574)

**Type:** other: Benzene leukemogenesis: an environmental carcinogen-induced tissue specific model of of neoplasia using genetically altered mouse models.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(376)

**Type:** other: Measurement and characterization of micronuclei in cultured primary lung cells of mice following inhalation exposure to benzene.

**Remark:** The genotoxic effects of benzene in lung cells of mice exposed to single acute doses by inhalation have been estimated by cytogenetic analysis of micronuclei in primary cultures of lung fibroblasts. Mice were nose-only exposed to 1000 ppm. for 30 or 60 min or to 3500 ppm. for 30 min and sacrificed 24 h after the end of exposure. Lung fibroblasts were cultured attached to coverslips for 72 h, the last 48 h in the presence of 0.75 microgram/ml cytochalasin B. Micronuclei were scored in binucleate cells. The mechanism(s) of micronucleus induction was characterized by immunofluorescent staining of kinetochore proteins (CREST staining), which allowed micronuclei due to chromosome loss (kinetochore-positive) to be distinguished from those produced by chromosome breakage (kinetochore-negative). Three- and 4-fold statistically significant increases in total micronucleus frequencies were observed in all

benzene-exposed mice with respect to unexposed controls. The effect was neither concentration nor time dependent. This is compatible with a plateau dose-effect relationship for the effects on bone marrow, which is explained by saturation of metabolism. Both chromosome loss and chromosome breakage appear to contribute to micronucleus formation, suggesting that in addition to chromosome rearrangements, aneuploidy may be a relevant early genotoxic event associated with benzene carcinogenicity. Under the same treatment conditions no micronucleus induction could be shown in spleen lymphocytes, suggesting that with very short benzene exposures cells at the first contact site with local metabolizing capacity have a higher probability of genetic alterations potentially leading to neoplasia.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

21-JUL-2000

(919)

**Type:** other: Benzene status assessment - priority substances program.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(763)

**Type:** other: Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure.

**Remark:** Potential additive effects of ethanol consumption, a common life-style factor, and low-level benzene exposure, a ubiquitous environmental pollutant, were investigated. Ethanol is a potent inducer of the cytochrome P-450 2E1 (CYP2E1) enzyme, which bioactivates benzene to metabolites with known genotoxicity and immunotoxicity. A liquid diet containing 4.1% ethanol was used to induce hepatic CYP2E1 activity by 4-fold in female CD-1 mice. Groups of ethanol-treated or pair-fed control mice were exposed to benzene or filtered air in inhalation chambers for 7 h/d, 5 d/wk for 6 or 11 wk. The initial experiment focused on immunotoxicity endpoints based on literature reports that ethanol enhances high-dose benzene effects on spleen, thymus, and bone marrow cellularity and on peripheral red blood cell (RBC) and white blood cell (WBC) counts. No statistically significant alterations were found in spleen lymphocyte cellularity, subtype profile, or function (mitogen-induced proliferation, cytokine production, or natural killer cell lytic activity) after 6 wk of ethanol diet, 0.44 ppm benzene exposure, or both. This observed absence of immunomodulation by ethanol alone, a potential confounding factor, further validates our previously established murine model of sustained CYP2E1 induction by dietary ethanol. Subsequent experiments involved a 10-fold higher benzene level for a longer time of 11 wk and focused on genotoxic endpoints in known target tissues. Bone marrow

and spleen cells were evaluated for DNA-protein cross-links, a sensitive transient index of genetic damage, and spleen lymphocytes were monitored for hprt-mutant frequency, a biomarker of cumulative genetic insult. No treatment-associated changes in either genotoxic endpoint were detected in animals exposed to 4.4 ppm benzene for 6 or 11 wk with or without coexposure to ethanol. Thus, our observations suggest an absence of genetic toxicity in CD-1 mice exposed to environmentally relevant levels of benzene with or without CYP2E1 induction.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

15-AUG-2000 (271)

**Type:** other: Benzene-induced genotoxicity.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002 (1253)

**Type:** other: T cell-dependent immune reactions to reactive benzene metabolites in mice.

**Remark:** Using the popliteal lymph node (PLN) assay in mice, we studied the sensitizing potential of benzene and its metabolites. Whereas benzene and phenol failed to induce a PLN reaction, catechol and hydroquinone induced a moderate, and p-benzoquinone a vigorous response. Following a single injection of the reactive metabolite p-benzoquinone (100 nmol/mouse), cellularity in the draining PLN was increased > 15-fold, and reverted back to normal only after approximately 100 days. Although the PLN response was T cell-dependent, flow cytometric analysis revealed that the increased cellularity in the PLN after a single injection of p-benzoquinone was mainly due to an increase in B cells. Mice primed to p-benzoquinone and challenged with a small dose of p-benzoquinone (0.1 nmol/mouse) mounted a secondary PLN reaction, indicating hapten specificity of the reaction; this was confirmed by results obtained in the adoptive transfer PLN assay. An unexpected finding was the secondary PLN response to benzene (1 nmol/mouse) observed in mice primed to p-benzoquinone. This finding suggests that some of the benzene (at least 10%) was locally converted into p-benzoquinone, which then elicited the secondary response observed. In conclusion, the reactive intermediate metabolites hydroquinone and p-benzoquinone can act as haptens and sensitize; their precursors, benzene and phenol, may be considered as prohaptens.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

24-JUL-2000 (340)

**Type:** other: Bioreactivity of glutathionyl hydroquinone with implications to benzene toxicity.

**Remark:** Glutathionyl hydroquinone (GHQ), a highly reactive metabolite of benzene, has been implicated as a causative intermediate of benzene toxicity. To substantiate, the bioreactivity of GHQ was investigated under in vitro and in vivo conditions using end points, characteristic of benzene toxicity. Under in vitro conditions, the presence of GHQ: (a) linearly increased the release of aldehydic products from L-glutamate or deoxyuridine at GHQ concentrations of 5 25 microM and from rat liver homogenates at GHQ concentrations of 50 250 microM; (b) cleaved plasmid pUC 18 supercoiled DNA through a single strand nick to yield open circular relaxed DNA, and through a double strand cut to give out linear DNA at GHQ concentrations of 25-200 microM, with evidence of protection by catalase and superoxide dismutase; and (c) induced cross-linking and polymerization of lymphocyte nuclear DNA through in situ generation of GHQ, which was protected by pretreatment of lymphocytes with N-ethylmaleimide. In vivo exposure of Swiss albino mice to GHQ (100 mg/kg, intraperitoneally once daily for 30 days) resulted in significant increase of liver weight and inhibition of mitotic index in the bone marrow. The other test parameters, namely spleen weight, hematological indices, hepatic sulphahydryl content and nonenzymatic lipid peroxidation, and chromosomal aberrations in the bone marrow were, however, unaffected by GHQ treatment. The observations indicate pro-oxidant and cytotoxic potential of GHQ, mediated by the reactive oxygen species generated during the course of its auto-oxidation. Bioreactivity of GHQ with cellular macromolecules in vitro and inhibition of mitotic index of bone marrow on in vivo exposure have relevance to benzene toxicity, although in situ generation of GHQ at the site of action appears critical in bringing about hematological and chromosomal effects that were probably spared due to rapid metabolic disposition and, consequently, poor bioavailability of intraperitoneally administered GHQ.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(13)

**Remark:** Benzene is an occupational hazard and environmental toxicant found in cigarette smoke, gasoline, and the chemical industry. The major health concern associated with benzene exposure is leukemia. Studies using microsomal preparations from human, mouse, rabbit, and rat to determine species differences in the metabolism of benzene to phenol, hydroquinone and catechol, indicate that the rat is most similar, both quantitatively and qualitatively, to the human in pulmonary microsomal metabolism of benzene. With hepatic microsomes, rat is most similar to human in metabolite formation at the two lower concentrations examined (24 and 200 microM), while at the two higher concentrations (700 and 1000 microM) mouse is most similar in phenol formation. In

all species, the enzyme system responsible for benzene metabolism approached saturation in hepatic microsomes but not in pulmonary microsomes. In pulmonary microsomes from mouse, rat, and human, phenol appeared to competitively inhibit benzene metabolism resulting in a greater proportion of phenol being converted to hydroquinone when the benzene concentration increased. The opposite effect was seen in hepatic microsomes. These findings support the hypothesis that the lung plays an important role in benzene metabolism, and therefore, toxicity.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

18-SEP-2000

(905)

**Type:** other: California perspective on the assessment of benzene toxicological risks.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(1306)

**Remark:** When characterizing the health risks for man by exposure to chemicals, species-specific differences have to be taken into consideration, otherwise extrapolation from animal data to the human situation would be inadequate. The site-specific toxicity of chemicals may be explained by the following alternatives: (1) reactive metabolites are generated in the liver and subsequently transported to the target tissue(s); (2) metabolism of the parent compound occurs in the target tissue, a pathway by which the enzymes necessary for activation must be expressed in the target tissue. Cytochrome P450 2E1 (CYP2E1) is an important phase-I enzyme activating several chemicals. In the study described in this paper, myeloid intra- and interspecies variability in the expression of CYP2E1 has been investigated in rats, rabbits and man, because the bone marrow represents an important target organ for toxic effects of several chemicals, e.g. benzene. CYP2E1 at the protein level was detected by Western blotting and enzyme activities were determined by CYP2E1-dependent hydroxylation of chlorzoxazone (CLX). In the bone marrow of Wistar rats, the CLX hydroxylase activities were within the same order of magnitude (range: 0.1-0.4 pmol/mg protein per min) as previously described for mice (range 0.2-0.8 pmol/mg protein per min), whereas the CYP2E1 activities in two strains of rabbits were significantly higher (range: 1.7-4.7 pmol/mg protein per min) than in the rodents ( $P < 0.05$ ). In human CD34+ bone marrow stem cells, CYP2E1 could also be detected on the protein level by Western blotting. The data demonstrate a presence of CYP2E1 in the bone marrow of all species investigated, thus supporting the hypothesis of CYP2E1-dependent local metabolism of several chemicals as a factor possibly contributing to their myelotoxicity and haematotoxicity. The data show that intraspecies/intrastrain

variability of CYP2E1 activity in rodents is small. However, CYP2E1 activity between rodents and a non-rodent species was quite different indicating considerable interspecies variability.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

18-SEP-2000

(98)

**Type:** other: Carcinogenic effects of benzene - a status update and research needs to improve risk assessment: U.S. EPA perspective.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(1079)

**Remark:** Although relatively rare, leukemias place a considerable financial burden on society and cause psychologic trauma to many families. Leukemia is the most common cancer in children. The causes of leukemia in adults and children are largely unknown, but occupational and environmental factors are strongly suspected. Genetic predisposition may also play a major role. Our aim is to use molecular epidemiology and toxicology to find the cause of leukemia and develop biomarkers of leukemia risk. We have studied benzene as a model chemical leukemogen, and we have identified risk factors for susceptibility to benzene toxicity. Numerous studies have associated exposure to benzene with increased levels of chromosome aberrations in circulating lymphocytes of exposed workers. Increased levels of chromosome aberrations have, in turn, been correlated with a heightened risk of cancer, especially for hematologic malignancy, in two recent cohort studies in Europe. Conventional chromosome analysis is laborious, however, and requires highly trained personnel. Further, it lacks statistical power, as only a small number of cells can be examined. The recently developed fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based technologies have allowed the detection of specific chromosome aberrations. These techniques are far less time consuming and are more sensitive than classical chromosomal analysis. Because leukemias commonly show a variety of specific chromosome aberrations, detection of these aberrations by FISH and PCR in peripheral blood may provide improved biomarkers of leukemia risk.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

18-SEP-2000

(1061)

**Type:** other: Biological monitoring of benzene in residents living near petrochemical industrial areas in Korea.

**Remark:** Residents who live near petrochemical industrial areas risk exposure to a variety of petrochemicals, including benzene and benzene-containing liquids. It is a serious concern because benzene is a human carcinogen naturally present in petroleum and gasoline. The aim of this study was to assess the exposure to benzene, measured by personal/indoor/outdoor air sampling, and to estimate the relationship between the air samples and biological monitoring data. Through biological monitoring, the authors investigated s-phenylmercapturic acid (s-PMA), minor urinary metabolites of benzene, and benzene in blood. The external benzene exposure of 115 subjects was measured with passive dosimeters and urinary s-PMA and blood-benzene were determined by GC/MS. The mean concentration of benzene in the breathing zone of residents was 6.3 microgram/m<sup>3</sup>, slightly higher than indoor or outdoor concentrations. Personal, indoor and outdoor concentrations of benzene were significantly correlated to each other. S-PMA was affected by personal exposure ( $p < 0.05$ ) and was differed according to age ( $p < 0.05$ ) and residence time ( $p < 0.05$ ). Blood benzene was not affected by external benzene during these periods.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

25-JUL-2000

(199)

**Type:** other: Effects of mixture of benzene, toluene and xylene on liver and renal functions in rats.

**Remark:** Effects of the mixt. of benzene, toluene and xylene on liver and renal functions in rats and the relationship between these effects and lipid peroxidn. (LPO) were studied. The results showed that the mixt. could induce defect in protein synthesis in liver cell and inhibit the transport and accumulation of bile acids by hepatocytes. The mixt. could enhance the permeability of parenchymal cells when benzene was the dominant component as detd. by measuring glutamic-oxaloacetic transaminase (AST), glutamic-pyruvic transaminase (ALT) and lactic dehydrogenase (LDH) in serum. The mixt. could induce LPO in rats by subacute exposure by measuring the contents of serum malondialdehyde (MDA), and could inhibit the activity of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The contents of serum protein were neg. correlated with the contents of serum MDA, and pos. correlated with the activity of serum SOD. The contents of serum albumin were pos. correlated with the activity of serum SOD and GSH-Px, the contents of serum bile acids were neg. correlated with the activity of serum SOD. The increase in the level of LPO and the decrease in the activity of serum SOD and GSH-Px may contribute to the mixt.- mediated hepatotoxicity. When xylene was the dominant, the mixt. might injure the renal function in rats.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
13-FEB-2002 (1229)

**Type:** other: Experimental studies of mixed benzene exposure on blood system, blood levels of lipid and lipid peroxidation in rats.

**Remark:** Objective: To study sub-acute effects of mixed benzene exposure on blood system, blood levels of lipid and lipid peroxidn. and activity of anti-oxidase in rats.

Methods: Male SD rats were divided randomly into five groups, control group with corn oil, group B with 5 mg of benzene (BZ) plus 44 mg of toluene (Tol) plus 500 mg xylene (Xyl) per kg, group A1 with 440 mg of BZ plus 88 mg of Tol plus 22 mg of Xyl per kg, group A2 with one fourth dose as group A1, and group A3 with one fourth of dose as group A2, via peritoneal injection once every other day for 18 times.

Results: As compared with control group, blood lymphocyte percentage decreased significantly and blood monocyte count and monocyte percentage, granulocyte count and granulocyte percentage increased significantly in groups A1 and B; the serum total cholesterol (TC) and triglyceride (TG) increased significantly in group A1; the serum level of TG correlated inversely with activity of superoxide dismutase (SOD) ( $r = -0.3135$ ,  $P < 0.05$ ), serum TC correlated with MDA ( $r = 0.2909$ ,  $P < 0.05$ ), serum level of malonyl dialdehyde (MDA) increased significantly and serum activity of GSH-Px decreased significantly in all exposed groups. Bone marrow levels of MDA in group A1 and activity of glutathione peroxidase (GSH-Px) in groups A1 and A2 were significantly higher than those in the control group ( $P < 0.05$ ).

Conclusion: The above results showed that subacute exposure to mixed benzene could cause change in white blood cell differential count, increase lipid peroxidn., and compensatively increase activities of SOD and GSH-Px. Enhancement of lipid peroxidn. and decrease in serum activity of SOD were important causes for increase in blood lipid level.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
13-FEB-2002 (1230)

**Type:** other: Hydroquinone, a reactive metabolite of benzene, inhibits NF-kappa B in primary human CD4+ T lymphocytes.

**Remark:** Hydroquinone (HQ), a reactive metabolite of benzene, is present in cigarette smoke and is known to inhibit mitogen-stimulated activation of both T and B lymphocytes. Despite extensive study, the underlying mechanism for HQ's immunotoxicity is not clear. NF-kappa B is a transcription factor known to regulate the expression of a number of genes critical for normal T cell activation. We therefore hypothesized that NF-kappa B might be involved in HQ-induced

immunosuppression. In this study, we demonstrate that 1 microM HQ inhibits tumor necrosis factor alpha induced activation of NF-kappa B in primary human CD4+ T cells. This inhibition is not accompanied by a loss in viability, and HQ-treated T cells maintain other active signaling pathways throughout the exposure duration. Additionally, the inhibition of NF-kappa B is reversible as HQ-treated T cells regain normal functioning after 72 h in culture. HQ does not appear to alter NF-kappa B directly as preincubation of nuclear extracts with HQ does not diminish activity of this protein. We further demonstrate that 1 microM HQ inhibits intracellular IL-2 production in T cells stimulated with phorbol ester but does not alter surface expression of CD25 (the alpha-subunit of the IL-2 receptor). These data suggest that NF-kappa B may be an important molecular mediator of HQ's (and benzene's) immunotoxicity.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(912)

**Type:** other: Hydroquinone inhibits PMA-induced activation of NF kappa B in primary human CD19 SUP + B lymphocytes.

**Remark:** Hydroquinone (HQ), a reactive metabolite of benzene, is known to inhibit mitogen-stimulated activation of both T and B lymphocytes. Despite extensive study, the underlying mechanism for the immunotoxicity of the HQ is not clear. We have previously demonstrated that 1 mu mol/L HQ inhibits TNF-induced activation of NFkB in CD4 SUP + T cells, resulting in decreased IL-2 production. NFkB, known to be important in T lymphocytes, also plays a critical role in normal B cell development and activation. We therefore hypothesized that alterations in NFkB might be involved in HQ-induced B cell immunosuppression as well. In this study, we demonstrate that 1-10 mu mol/L HQ inhibits PMA/ionomycin-induced activation of NFkB in primary human CD19 SUP + B cells. Inhibition of NFkB is accompanied by a dose-dependent decrease in PMA-stimulated production of TNF with no corresponding loss in viability or increased apoptosis. HQ also does not appear to alter NFkB directly, as preincubation of B cell nuclear extracts with HQ does not diminish DNA binding activity of this protein. In contrast to T cells, inhibition of NFkB by HQ in B cells is not reversible after 72 h in culture, suggesting a long-term functional suppression. These data support our original findings in T cells and indicate that NFkB is particularly susceptible to inhibition by HQ. We further hypothesize that inhibition of NFkB in lymphocytes, and perhaps other cell types as well, may play a significant role in the observed toxicity of HQ.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(913)

**Type:** other: The benzene metabolites hydroquinone and catechol act in synergy to induce dose-dependent hypoploidy and -5q31 in a human cell line.

**Remark:** Chronic exposure to high concentrations of benzene is associated with an increased incidence of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML). Studies of patients occupationally exposed to benzene show a pattern of cytogenetic aberrations involving loss of all or part of chromosomes 5 and/or 7 as well as trisomy 8 and we have previously reported that hydroquinone (HQ) induces deletions of 5, 7 and 8. Benzene metabolism is a requirement for bone marrow toxicity and the phenolic metabolites, HQ and catechol (CAT), have been implicated in benzene hematotoxicity. A research project was designed to determine whether CAT by itself and in conjunction with HQ could directly induce loss of chromosome 5 and/or 7 and gain of chromosome 8. Using fluorescence in situ hybridization with chromosome-specific 5, 7, and 8 probes we demonstrate that 5 to 150 uM CAT does not produce chromosomal aberrations, however CAT and 25 uM HQ can act in synergy to induce dose dependent loss of these chromosomes. In addition HQ/CAT selectively induces -5q which is not observed for HQ only. These results demonstrate for the first time that CAT/HQ act in synergy to induce specific chromosome loss found in secondary MDS/AML.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

24-JUL-2000

(1090)

**Type:** other: Loss of p53 in benzene-induced thymic lymphomas in p53+/- mice: evidence of chromosomal recombination.

**Remark:** The purpose of this study was to examine the role of chromosomal recombination in mediating p53 loss in benzene-induced thymic lymphomas in C57BL/6-Trp53 haplo-insufficient (N5) mice (p53+/- mice). The authors characterized loss of heterozygosity (LOH) on chromosome 11 using 7 microsatellite markers in 27 benzene-induced and 6 spontaneous thymic lymphomas. Eleven patterns of LOH were found between the induced and spontaneous tumors, with only 1 pattern being in common between the tumor groups. Nearly 90% (24 of 27) of benzene-induced tumors exhibited loss of the functional p53 allele locus, and 83% (20 of 24) of these tumors retained 2 copies of the disrupted p53 allele. Thus, benzene induces a high frequency of LOH on chromosome 11 in p53+/- mice, likely mediated by aberrant chromosomal recombination.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

04-JUL-2005

(130)

**Type:** other: The benzene metabolite, hydroquinone, selectively induces 5q31- and -7 in human CD34+CD19- bone marrow cells.

**Remark:** OBJECTIVE: Chronic exposure to high concentrations of benzene is associated with an increased incidence of myelodysplastic syndrome and acute myelogenous leukemia. Acute myelogenous leukemia developing in patients treated with alkylating agents for other cancers or occupationally exposed to benzene exhibit a pattern of cytogenetic aberrations predominantly involving loss of all or part of chromosomes 5 and/or 7. In contrast, trisomy 8 is observed equally in both de novo and secondary acute myelogenous leukemia. Studies using peripheral lymphocytes or lymphoblastoid cell lines have observed dose-dependent loss of chromosomes 5, 7, and 8 following treatment with the benzene metabolite, hydroquinone. The purpose of this study was to determine the dose response and specificity of hydroquinone-induced aberrations on chromosomes 5, 7, and 8 using human CD34+CD19 bone marrow cells. MATERIALS AND METHODS: Fluorescence in situ hybridization analysis was performed on CD34+CD19- bone marrow cells using the locus-specific probes, 5q31, 5p15.2, and centromeric probes specific for human chromosomes 7 and 8 following hydroquinone exposure. RESULTS: Hydroquinone exposure results in -7, selective deletion of 5q31 but not chromosome 5 and no loss or gain of chromosome 8 in human CD34+CD19- cells. CONCLUSION: CD34+ bone marrow cells are more susceptible and show a different pattern of cytogenetic aberrations as a result of hydroquinone exposure compared to lymphocytes. CD34+ bone marrow cells exhibit unique susceptibility to the development of specific chromosome aberrations that have been identified as the earliest structural changes occurring in the development of secondary myelodysplastic syndrome and acute myelogenous leukemia.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(1089)

**Type:** other: Genetic effects of petroleum fuels: II. Analysis of chromosome loss and hyperploidy in peripheral lymphocytes of gasoline station attendants.

**Remark:** Molecular cytogenetic methods were applied to investigate the effect of the occupational exposure to low concentrations of benzene and petroleum fuels on genomic stability. Twelve male gasoline station attendants (average benzene exposure of 0.32 mg/m<sup>3</sup> as 8h TWA) and 12 age- and smoking-matched unexposed controls were selected for the study. The incidence of hyperploidy and polyploidy in peripheral lymphocytes was evaluated through in situ hybridization of interphase cells, harvested 24 hr after stimulation, with centromeric probes of chromosomes 7, 11, 18, and X. For half of the subjects, metaphases harvested 24 hr later were analyzed. The incidence of chromosome loss in vitro was determined in cytokinesis-blocked cells, harvested at 66 hr, through the hybridization of micronuclei with a

pancentromeric probe. Ten thousand chromosomes (more than 200 metaphases equivalent) and 2,000 binucleated cells/person were scored for hyperploidy and micronucleus analysis, respectively. The results obtained did not show any exposure-related excess of hyperploidy or micronucleus formation. Conversely, the age of the subjects was significantly correlated with several markers of genomic instability, such as the incidence of chromosome X and chromosome 18 hyperploidy, total hyperploidy and polyploidy, and close to statistical significance with chromosome loss. Smoking habits did not appear to contribute significantly to the effects measured. The parallel analysis of hyperploidy and polyploidy in interphase nuclei in 24-hr cultures and in metaphase cells harvested 24 hr later showed basically similar incidences of aneuploid cells, indicating that no significant selection against hyperploid and polyploid types occurred during the first cell cycle in vitro.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

24-JUL-2000 (175)

**Type:** other: Molecular models of benzene leukemogenesis.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002 (569)

**Type:** other: Needs for research on benzene metabolism and dosimetry.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002 (1015)

**Type:** other: Presence of antibodies to heat stress proteins in workers exposed to benzene and in patients with benzene poisoning.

**Remark:** Heat shock or stress proteins (Hsps) are a group of proteins induced by a large number of xenobiotics, many of which are common in the working and living environment. The biological significance of the presence of antibodies against Hsps in humans is presently unknown. In the present study, 112 workers were selected and divided into four groups on the basis of their level of occupational exposure to benzene: a control group, two groups of workers exposed to either low (< 300 mg/m<sup>3</sup>) or high concentrations of benzene (> 300 mg/m<sup>3</sup>) and a group of workers who had experienced benzene poisoning. Blood samples from these workers were assayed for the number of peripheral white blood cells, concentration of hemoglobin, activities of serum superoxide dismutase (SOD), lymphocyte DNA damage and finally for the presence of antibodies to different human heat-shock proteins (Hsp27, Hsp60, Hsp71 and Hsp90). Benzene-poisoned workers showed a

high incidence of antibodies against Hsp71 (approximately 40%) which was associated with a decrease in white blood cells [ $3.84 \pm 1.13 \times 10^9$  versus  $7.68 \pm 1.84 \times 10^9$  in controls] and with an increase in activities of serum SOD ( $138.43 \pm 23.15$  micro/ml) and lymphocyte DNA damage (18.7%). These data suggest that antibodies against Hsps can potentially be useful biomonitoring tools to assess if workers are experiencing or have experienced abnormal xenobiotic-induced stress within their living and working environment.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (2) valid with restrictions

21-JUL-2000

(1283)

**Type:** other: Overview of the toxicology of benzene.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(1106)

**Type:** other: Possible mechanisms of carcinogenesis after exposure to benzene.

**Remark:** This review covers the history of the toxicology of benzene, current exposure levels, the metabolism of benzene, reactions of the metabolites with biomolecules, and possible mechanisms of carcinogenesis due to benzene. Epidemiological evidence indicates a relationship between exposure to benzene and the occurrence of acute non-lymphocytic leukaemia in humans. Working groups convened by IARC and other organizations have therefore judged that there is sufficient evidence for classifying benzene as a human carcinogen. Despite much research, including numerous studies in animals, the detailed mechanism of the carcinogenicity of benzene is unknown. The significant differences in the responses of rodents and humans to benzene are not understood. Benzene forms many metabolites, some of which are reactive towards biomolecules, but the metabolite(s) responsible for the induction of leukaemia is unknown. Candidate metabolites, either singly or in combination, include epoxides, oxepins, quinones and aldehydes, all of which are reactive towards proteins and DNA. Our studies on muconaldehydes and benzene oxide-oxepin are discussed in this context. The significance of DNA adduct formation in respect of human leukaemia is uncertain. The overall reactivity of benzene towards DNA has been shown to be very low in experimental animals, although dose-related reactivity of metabolites with DNA was observed. The lack of significant DNA reactivity is reflected in the lack of activity of benzene in short-term tests for genotoxicity; however, benzene causes oxidative stress, which can be detected as oxidative damage to DNA. Mechanisms other than DNA damage may play a role in benzene-related toxicity, e.g. reactions of benzene metabolites with essential enzymes such as topoisomerase II.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

04-JUL-2005

(420)

**Type:** other: Physiologically based modeling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures.

**Remark:** The objective of this study was to predict and validate the theoretically possible, aximal impact of metabolic interactions on the blood concentration profile of each component in mixtures of volatile organic chemicals (VOCs) (dichloromethane (DCM), benzene (BEN), trichloroethylene (TCE), toluene (TOL), tetrachloroethylene (PER), ethylbenzene (EBZ), styrene (STY), as well as para, ortho-, and meta-xylene (p-XYL, o-XYL, m-XYL)) in the rat. The methodology consisted of: (1) obtaining the validated, physiologically based toxicokinetic (PBTK) model for each of the mixture components from the literature, (2) substituting the Michaelis-Menten description of metabolism with an equation based on the hepatic extraction ratio (E) for simulating the maximal impact of metabolic interactions (i.e., by setting E to 0 or 1 for simulating maximal inhibition or induction, respectively), and (3) validating the PBTK model simulations by comparing the predicted boundaries of venous blood concentrations with the experimental data obtained following exposure to various mixtures of VOCs. All experimental venous blood concentration data for 9 of the 10 chemicals investigated in the present study (PER excepted) fell within the boundaries of the maximal impact of metabolic inhibition and induction predicted by the PBTK model. The modeling approach validated in this study represents a potentially useful tool for screening/identifying the chemicals for which metabolic interactions are likely to be important in the context of mixed exposures and mixture risk assessment.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

13-FEB-2002

(462)

**Remark:** Two human carcinogens that have been extensively studied are vinyl chloride and benzene. The active metabolites used in this study are chloroacetaldehyde (CAA) and para-benzoquinone (pBQ). Each forms exocyclic adducts between the N1 and N6 of A, the N3 and N4 of C and the N1 and N2 of G. Only CAA has been found to form the N2,3 adduct of G. CAA and pBQ adducts differ structurally in size and in the number of added rings, pBQ adding two rings to the base, while etheno bases have a single five-membered ring. The mechanism of repair of these two types of adducts by human enzymes has been studied in our laboratory with defined oligodeoxynucleotides and a site-specific adduct. The etheno derivatives are repaired by DNA glycosylase activity; two mammalian glycosylases are responsible: alkylpurine-DNA-N-glycosylase (APNG) and mismatch-specific thymine-DNA glycosylase. The former repairs 1,N6-ethenoA

(epsilon A) as rapidly as the original substrate, 3-methyladenine, while the latter repairs 3,N4-ethenoC (epsilon C) more efficiently than the G/T mismatch. Our finding that there are separate enzymes for epsilon A and epsilon C has been confirmed by the use of tissue extracts from an APNG knockout mouse. As pBQ is much less efficient than CAA in modifying bases, the biochemical studies required total synthesis of the nucleosides. Furthermore, the pBQ adduct-containing oligomers are cleaved, to various extents by a different class of enzyme: human and bacterial N-5'-alkylpurine (AP) endonucleases. The enzyme incises such oligomers 5' to the adduct and generates 3'-hydroxyl and 5'-phosphoryl termini but leaves the modified base on the 5'-terminus of the 3' cleavage fragment ('dangling base'). Using active-site mutants of the human AP endonuclease, we found that the active site for the primary substrate, abasic (AP) site, is the same as that for the bulky pBQ adducts. There appears to be no clear rationale for the widely differing recognition and repair mechanisms for these exocyclic adducts, as shown for the repair of the same types of modification on different bases (e.g. epsilon A and epsilon C) and for completely unrelated lesions (e.g. AP site and pBQ adducts). Another important variable that affects the rate and extent of repair is the effect of neighbouring bases. In the case of epsilon A, this sequence-dependent repair correlates with the extent of double-strandedness of the substrate, as demonstrated by thermal stability studies.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

04-JUL-2005

(1038)

**Type:** other: Measurement of benzene in the workplace and its evolution process.

**Remark:** Benzene and its measurement continues to be important in the field of industrial hygiene. Part I of this review provides an overview and details of the methods used in the past; history of the American Conference of Governmental Industrial Hygienists' threshold limit values for benzene; and reviews portable, grab, and integrated sampling methods as well as the various analytical methods. It is important to review and understand the past in order to predict future trends. Part II of this review provides a discussion of currently accepted methodology and possible future happenings regarding measurement of benzene in workplaces. The gap between occupational and environmental monitoring is becoming narrow. Environmental levels will always be lower than the occupational, but as the push for lower threshold limit values continues, the focus should be on the environmental aspect as the ultimate limiting factor with respect to measurement. The charcoal tube/carbon disulfide desorption procedure is slowly being stretched to its limit with respect to benzene. It may be time for serious consideration in North America regarding adoption of the proven European procedure of thermal desorption using a

- porous polymer tube for analysis of benzene.  
**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction
- 24-JUL-2000 (1209) (1210)
- Type:** other: Recent developments in benzene risk assessment.
- Remark:** No abstract given in paper.  
**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey
- 13-FEB-2002 (590)
- Type:** other: Dermal absorption of benzene: implications for work practices and regulations.
- Remark:** Because the risk of leukemia for workers exposed to 1 ppm of benzene for 40 years is estimated to be 70% greater than the risk for unexposed persons, the National Institute for Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) recommend that the allowable airborne exposure level be 0.1 ppm. Using an experimentally determined dermal flux (permeability) value for benzene through skin, the authors calculated the amount of benzene absorbed through a known surface area (e.g., hands) during exposures where solvents contaminated with benzene were used for cleaning. Even at current contamination levels, which are less than 0.1% in most products, the amount of benzene absorbed through the skin over a long period can be significant, depending on exposure time and exposed skin surface areas. In the example given, the risk for leukemia was increased by 42%. Therefore, the authors recommend that the liquid benzene concentration that triggers labeling, worker education, and protective measures to minimize skin exposure be reduced from 0.1% to 0.01%.
- Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction
- 21-JUL-2000 (596)
- Type:** other: Structure-activity relationships in the induction of DNA-protein cross-links by hematotoxic ring-opened benzene metabolites and related compounds in HL60 cells.
- Remark:** Previous studies from the authors' lab. have demonstrated the formation of DNA-protein crosslinks (DNAPC), a potentially cytotoxic and genotoxic lesion induced by many leukemogenic agents, in bone marrow cells of mice administered benzene, however, reactive benzene metabolites involved in DNAPC formation by benzene have not been characterized. The present studies examd. DNA PC formation in HL60 cells treated with trans,trans-muconaldehyde (MUC), a hematotoxic ring-opened metabolite of benzene, as well as with MUC metabolites and structurally related compds. Using a K+/SDS pptn. assay for DNAPC detn., concn.- and time-dependent increases in DNAPC formation were obsd. 2 and

4 h after treatment of HL60 cells with 50, 75 and 100 microM MUC. No increases in DNAPC levels were measured in HL60 cells 4 h after treatment with the MUC metabolites 6-hydroxy-trans,trans-2,4-hexadienal (HO-M-CHO), 6-oxo-trans,trans-2,4-hexadienoic acid (CHO-M-COOH), or trans,trans-muconic acid (HOOC-M-COOH), each at 100 microM. Significant increases in DNAPC levels were obsd. 4 h after treatment with 500 and 1000 microM HO-M-CHO, but not CHO-M-COOH. No effect on DNAPC levels was obsd. 4 h after treatment with 100 microM for trans,trans-2,4-hexadienal, trans-2-hexenal, hexanal, trans,trans-2,4-hexadiene, glutaraldehyde, or acrolein. DNAPC induced by MUC and HO-M-CHO may be cytotoxic lesions, as increases in DNAPC levels by these compds. correlated with decreases in cell viability. Except for acrolein, compds. not inducing DNAPC at 100 microM also did not affect cell viability. These studies suggest that both aldehydic carbons of MUC contribute to DNAPC induction, and that the presence of alpha,beta-unsatd. double bonds conjugated with the aldehyde groups increases the ability of MUC to induce DNAPC relative to the satd. dialdehyde glutaraldehyde.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(1026)

**Type:** other: Benzene percutaneous absorption: dermal exposure relative to other benzene sources.

**Remark:** Skin is one of several exposure routes whereby benzene, a widely distributed environmental contaminant that causes leukemia, enters the body, so accurate predictions of its percutaneous absorption are important for risk assessment. Determining benzene's skin-exposure dose and subsequent absorption is difficult because it has a low boiling point and exists as both liquid and vapor. Industrial and environmental benzene is present as a contaminant in other vehicles/solvents, and its percutaneous absorption is in part dependent upon co-solvent volatility. Co-solvents such as benzene in toluene rapidly evaporate from skin, whereas benzene contaminant in water is retained on skin longer due to water's lower volatility. Co-solvents can also affect benzene-skin partition coefficients; thus, permeability coefficients and percentage doses absorbed can vary many-fold. The exposure situation will determine percutaneous absorption, which, if low, can be overwhelmed by benzene intake from the food we eat and the air we breathe.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

21-JUL-2000

(1246)

**Type:** other: Study on absorption of environmental contaminants in low-level exposure by pharmacokinetic analysis.

**Remark:** A dynamic generating toxic gas system and a nose-only exposure system were used for the pharmacokinetic study of inhaled environmental contaminants such as benzene, toluene, xylene, ethylbenzene, chlorobenzene, styrene, isopropylbenzene, tetrachloroethylene, nonane, and methylcyclohexane in the male guinea pig. The change of these substances in blood with time was detd. simultaneously by solid phase microextn. (SPME)-gas chromatog. (GC). The fraction of absorption of benzene at low (121 microgram/m3) exposure was 4.8 times higher than that at high (12.1 mg/m3) exposure. The pharmacokinetics of these substances was evaluated by using linear compartment models. More styrene was absorbed than tetrachloroethylene at low exposure. The metabolic elimination of these compds. at various exposure concns. was extrapolated by using estd. pharmacokinetic parameters. The results showed that the exposure concns. in gas for all chems. were equal, and the differences in absorption quantities and metabolic elimination rates may be simultaneously considered in evaluation of potential risk assessment. The fundamental data used for risk assessment were presented.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(480)

**Type:** other: Development of liquid chromatography-electrospray ionization-tandem mass spectrometry methods for determination of urinary metabolites of benzene in humans.

**Remark:** To investigate the ways in which different levels of exposure affect the metabolic activation pathways of benzene in humans, and to examine the relationship between urinary metabolites and other biological markers, we have developed two sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays for quantitation of the benzene metabolites trans,transmuconic acid (t,t-MA), S-phenylmercapturic acid (S-PMA), hydroquinone (HQ), catechol (CAT), and for estimation of 1,2,4-trihydroxybenzene (BT). In our first assay, urinary S-PMA and t,t-MA were measured simultaneously by liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring (LC-ESI-MS/MS-SRM) in the negative ionization mode. In this assay, the metabolites [13C6]-S-PMA and [13C6]-t,t-MA were used as internal standards. The efficacy of this specific assay was evaluated in human urine specimens from 28 smokers and 18 nonsmokers serving as the benzene-exposed and nonexposed groups, respectively. The coefficient of variation (CV) of analyses on different days (n = 8) for S-PMA was 7% for samples containing 9.4 micrograms/L urine, and for t,t-MA was 10% for samples containing 0.07 mg/L. The mean levels of S-PMA and t,t-MA in smokers were 1.9-fold (p = 0.02) and 2.1-fold (p = 0.03) higher, respectively, than those in nonsmokers.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

21-JUL-2000

(765)

**Type:** other: The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues.

**Remark:** No abstract given in the paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(961)

**Type:** other: Recent developments in the understanding of benzene toxicity and leukemogenesis.

**Remark:** This review (with 57 references) summarizes recent developments in benzene toxicity and leukemogenesis including: (1) description of adverse effects of benzene and suggested dose relationships; (2) benzene metabolism; (3) factors which contribute to the mechanism of benzene toxicity; (5) DNA adducts; (6) reactive oxygen species; (7) impact on myeloid differentiation and maturation; (8) susceptibility to benzene toxicity. Sensitivity to benzene may result from a series of polymorphisms in enzymes which modulate the production of toxic benzene metabolites. Among the factors which influence benzene toxicity are: (1) polymorphisms of cytochrome P450 2E1 (CYP 2E1) activity because it is the major enzyme involved in benzene hydroxylation; (2) glutathione (GSH) transferase activity; (3) comparative myeloperoxidase activity in bone marrow; (4) NAD(P)H:quinone oxidoreductase (NQO1) activity in bone marrow. An individual's susceptibility to benzene toxicity may be determined by variability in these sensitivity factors. Thus, the high activity of CYP 2E1 would increase the rate of formation of benzene metabolites and thereby render the individual more susceptible to benzene toxicity. However, depending on the activity of the other three enzymes, the influence of any one of the other enzymes could be modulated. Thus, a high level of NQO1 might reduce the significance of high CYP 2E1. Taken together, this may indicate that high CYP 2E1 and low GSH transferase in the liver and low NQO1 coupled with high myeloperoxidase activity in the bone marrow of an individual would render that person more sensitive to benzene toxicity than someone having the reverse levels of enzymatic activity. Other factors relating to differentiation and maturation of bone marrow cells may also contribute to sensitivity but these have yet to be defined.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(1067)

**Type:** other: Utility of a mouse model for studying the effects of benzene on the myeloid lineage: effects of hydroquinone on a model myeloid system.

**Remark:** No abstract given in paper.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(595)

**Type:** other: Dose-dependent metabolism of benzene in hamsters, rats, and mice.

**Remark:** The disposition of oral doses of [14C]benzene was investigated using a range of doses that included lower levels (0.02 and 0.1 mg/kg) than have been studied previously in rat, mouse, and in hamster, a species which has not been previously examined for its capacity to metabolize benzene. Saturation of metabolism of benzene was apparent as the dose increased, and a considerable percentage of the highest doses (100 mg/kg) was exhaled unchanged. Most of the remainder of the radioactivity was excreted as metabolites in urine, and significant metabolite-specific changes occurred as a function of dose and species. Phenyl sulfate was the predominant metabolite in rat urine at all dose levels (64-73% of urinary radioactivity), followed by prephenylmercapturic acid (10-11%). Phenyl sulfate (24-32%) and hydroquinone glucuronide (27-29%) were the predominant metabolites formed by mice. Mice produced considerably more muconic acid (15%), which is derived from the toxic metabolite muconaldehyde, than did rats (7%) at a dose of 0.1 mg/kg. Unlike both rats and mice, hydroquinone glucuronide (24-29%) and muconic acid (19-31%) were the primary urinary metabolites formed by hamsters. Two metabolites not previously detected in the urine of rats or mice after single doses, 1,2,4-trihydroxybenzene and catechol sulfate, were found in hamster urine. These data indicate the hamsters metabolize benzene to more highly oxidized, toxic products than do rats or mice.

Additional Comments: This is the first report that hamsters metabolize a significantly greater portion of a dose of benzene to muconic acid than do either rats or mice. The relative amounts of muconic acid eliminated were also dose dependent, accounting for approximately 19% of the high dose versus 30% of the low dose excreted in the urine. Hamsters also metabolized as much of each benzene dose to hydroquinone as did mice and, like mice, excreted it primarily as the glucuronide conjugate. Additionally, hamsters, but not rats or mice, produced detectable amounts of trihydroxybenzene and catechol sulfate. These observations suggest that if muconaldehyde, hydroquinone, catechol and trihydroxybenzene are, as speculated by many investigators, among the most toxic metabolites of benzene and act synergistically, then the hamster should be one of the most sensitive animal models for studies of benzene toxicity. The hamster may serve as an appropriate model for

the most sensitive, high CYP 2E1 activity, humans and may be a useful model to provide better estimates of the degree to which muconaldehyde and other metabolites account for benzene toxicity and/or carcinogenicity.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(747)

**Type:** other: Utilization of breath analysis for exposure estimates of benzene associated with active smoking.

**Remark:** Three different expts. for benzene exposures assocd. with active smoking were carried out. In the first expt., the mean exhaled breath benzene concns. measured 1 min after an active smoke ranged from 58.1-81.3 microgram/m<sup>3</sup>, depending on the com. cigarette brand, while those measured prior to an active smoke ranged from 15.9-19.2 microgram/m<sup>3</sup>. The postexposure breath concns. were much higher than the mean breath concns. reported by some previous studies whose exposure conditions and postsampling times were not controlled. Similar to some previous decay studies conducted for different volatile org. compds. in different microenvironments, the second expt. showed that there was a rapid fall in the breath concn. and thereafter the decrease was much slower. One-compartment half-lives ranged from 30.1-57.8 min. Two-compartment half-lives ranged from 3.2-25.7 min for the first half-life and from 67-462 min for the second half-life. In the final repeated smoke expt. conducted with two specified time intervals, the breath concns. showed increasing trends for both the pre- and the postexposure concns., with few exceptions. However, none of the changes were statistically significant at P<0.05.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(584)

**Type:** other: Environmental. Aerobic biodegradation of benzene, toluene and ethylbenzene in liquid medium by a bacterial consortium, isolated from non-history clay soil, and their interrelation effect.

**Remark:** The present study was carried out in order to investigate the ability of isolated subsurface bacteria, from a non-history clay soil, to biodegrade the non-aqueous phase-liquids (NAPLs), monoaromatic hydrocarbons: benzene, toluene and ethylbenzene. First stage of the study was focussed on stand-alone biodegradation of each contaminant under described conditions. Benzene (100, 260 and 500 mg/l) exposed to isolated soil bacteria for 14 days, was biodegraded 100, 70 and 50%, respectively, ethylbenzene (100, 260 and 500 mg/l) at 85, 87 and 90%, respectively and toluene (100, 260 and 500 mg/l) revealed the lowest rate of 45, 50 and 52%. Toluene and ethylbenzene showed a direct increase in biodegradation associated with increase in their concentration. The second stage was the biodegradation of benzene, toluene and ethylbenzene admixture (all three

compounds at the very same concentrations, w/v) in glucose absence and supplemented with Tween 80 (10 and 15 mg/l). The overall biodegradation improved when contaminants were mixed together.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(43)

**Type:** other: Role of quinones in toxicology (review).

**Remark:** Quinones represent a class of toxicological intermediates which can create a variety of hazardous effects in vivo, including acute cytotoxicity, immunotoxicity, and carcinogenesis. The mechanisms by which quinones cause these effects can be quite complex. Quinones are Michael acceptors, and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. Alternatively, quinones are highly redox active molecules which can redox cycle with their semiquinone radicals, leading to formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins, and DNA. Formation of oxidatively damaged bases such as 8-oxodeoxyguanosine has been associated with aging and carcinogenesis. Furthermore, ROS can activate a number of signaling pathways, including protein kinase C and RAS. This review explores the varied cytotoxic effects of quinones using specific examples, including quinones produced from benzene, polycyclic aromatic hydrocarbons, estrogens, and catecholamines. The evidence strongly suggests that the numerous mechanisms of quinone toxicity (i.e., alkylation vs oxidative stress) can be correlated with the known pathology of the parent compound(s).

Additional Comments: This review covers the benzene-derived quinones that may play a role in bone marrow toxicity and discusses potential reactive intermediates as well as mechanistic and cellular factors [e.g., NADD(P)H:quinone oxidoreductase (NQO1), GSH] involved in metabolic activation, detoxification and toxicity of benzene.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (1) valid without restriction

24-JUL-2000

(132)

**Type:** other: Analysis and evaluation of trans,trans-muconic acid as a biomarker for benzene exposure (review).

**Remark:** Benzene is an important industrial chemical and, due to its occurrence in mineral oil and its formation in many combustion processes, a widespread environmental pollutant. Since benzene is hematoxic and has been classified as a human carcinogen, monitoring and control of benzene exposure is of importance. Although trans,trans-muconic acid (ttMA)

was identified as a urinary metabolite of benzene at the beginning of this century, only recently has its application as a biomarker for occupational and environmental benzene exposure been investigated. The range of metabolic conversion of benzene to ttMA is about 2-25% and dependent on the benzene exposure level, simultaneous exposure to toluene, and probably also to genetic factors. For the quantitation of ttMA in urine, HPLC methods using UV and diode array detection as well as GC methods combined with MS or FID detection have been described. Sample pretreatment for both HPLC and GC analysis comprises centrifugation and enrichment by solid-phase extraction on anion-exchange sorbents. Described derivatization procedures prior to GC analysis include reaction with N,O-bis(trimethylsilyl)acetamide, N,O-bis(trimethylsilyl)trifluoroacetamide, pentafluorobenzyl bromide and borontrifluoride-methanol. Reported limits of detection for HPLC methods range from 0.1 to 0.003 mg/L), whereas those reported for GC methods are 0.03-0.01 mg/L. Due to its higher specificity, GC methods appear to be more suitable for determination of low urinary ttMA levels caused by environmental exposure to benzene. In studies with occupational exposure to benzene (>0.1 ppm), good correlations between urinary ttMA excretion and benzene levels in breathing air are observed. From the reported regressions for these variables, mean excretion rates of ttMA of 1.9 mg/g creatinine or 2.5 mg/L at an exposure dose of 1 ppm over 8 h can be calculated. The smoking-related increase in urinary ttMA excretion reported in twelve studies ranged from 0.022 to 0.2 mg/g creatinine. Only a few studies have investigated the effect of exposure to environmental levels of benzene (<0.01 ppm) on urinary ttMA excretion. A trend for slightly increased ttMA levels in subjects living in areas with high automobile traffic density was observed, whereas exposure to environmental tobacco smoke did not significantly increase the urinary ttMA excretion. It is concluded that urinary ttMA is a suitable biomarker for benzene exposure at occupational levels as low as 0.1 ppm. Biomonitoring of exposure to environmental benzene levels (<0.01 ppm) using urinary ttMA appears to be possible only if the ingestion of dietary sorbic acid, another precursor to urinary ttMA, is taken into account.

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

24-JUL-2000

(1013)

**Type:** other: Environmental. Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer.

**Remark:** The addition of sulfate to an anaerobic petroleum-contaminated aquifer in which benzene was a major soluble contaminant resulted in removal of benzene from the groundwater. The loss in benzene was associated with a decrease in sulfate along the groundwater flow path,

relative to a conservative bromide tracer. Studies with (2-14C)-acetate and molybdate demonstrated that sulfate reduction was the predominant terminal electron-accepting process (TEAP) in the sulfate-amended sediments, and studies with (14C)benzene indicated that benzene oxidation was dependent upon sulfate reduction. Abundant ferrous iron in the subsurface likely prevented the generation of free sulfide in the groundwater during the field trial. Comparisons of benzene and sulfate depletion in the treatment zone indicated that benzene degradation could account for 53% of the sulfate depletion. These results suggest that the addition of sulfate stimulated the activity of benzene-degrading, sulfate-reducing microorganisms. This is the first field study demonstrating that it is possible to stimulate anaerobic benzene degradation in a petroleum-contaminated aquifer.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(32)

**Type:** other: Environmental. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1.

**Remark:** Although microbial growth on substrate mixts. is commonly encountered in bioremediation, wastewater treatment and fermn., math. modeling of mixed substrate kinetics has been limited. The kinetics of *Pseudomonas putida* F1 growing on benzene, toluene, phenol, and their mixts. is reported and math. models to describe these results are compared. The 3 aroms. can each act as C and energy sources for this strain. Biodegrdn. rates were measured in batch cultivations following a protocol that eliminated mass transfer limitations for the volatile substrates and considered the culture history of the inoculum and the initial substrate:inoculum mass ratio. Toluene and benzene were better growth substrates than phenol, resulting in faster growth and higher yield coeffs. In the concn. Ranges tested, toluene and benzene biodegrdn. kinetics were well described by the Monod model. The Monod model was also characterized phenol biodegrdn. by *P. putida* F1, although a small degree of substrate inhibition was noted. In mixt. expts., the rate of consumption of 1 substrate was obsd. to be affected by the presence of the others, although the degree of influence varied widely. Substrates were catabolized by the same enzymic pathway, but purely competitive enzyme kinetics did not capture substrate interactions well. Toluene significantly inhibited the biodegrdn. rate of both the other substrates; benzene slowed the consumption of phenol (but not of toluene). Phenol had little effect on the biodegrdn. of either toluene or benzene. Of the models tested, a sum kinetics with interaction parameters (SKIP) model provided the best description of paired substrate results. This model, with parameters detd. from 1- and 2-substrate expts., provided an excellent prediction of biodegrdn. kinetics for the 3-component mixt.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
14-FEB-2002 (928)

**Type:** other: Modulation of the toxicity and macromolecular binding of benzene metabolites by NAD(P)H:quinone oxidoreductase in transfected HL-60 cells.

**Remark:** Benzene is oxidized in the liver to produce a series of hydroxylated metabolites, including hydroquinone and 1,2,4-benzenetriol. These metabolites are activated to toxic and genotoxic species in the bone marrow via oxidation by myeloperoxidase (MPO). NAD(P)H:quinone oxidoreductase (NQO1) is an enzyme capable of reducing the oxidized quinone metabolites and thereby potentially reducing their toxicities. We introduced the NQO1 gene into the HL-60 cell line to create a high MPO-, high NQO1-expressing cell line, and tested its response in assays of benzene metabolite toxicity. NQO1 expression reduced a class of hydroquinone- and benzenetriol-induced DNA adducts by 79-86%. The cytotoxicity and apoptosis caused by hydroquinone were modestly reduced, while protein binding was unchanged and the rate of glutathione depletion increased. NQO1's activity in reducing a class of benzene metabolite-induced DNA adducts may be related to its known activities in maintaining membrane-bound endogenous antioxidants in reduced form. Alternatively, NQO1 activity may prevent the formation of adducts which result from polymerized products of the quinones. In either case, this protection by NQO1 may be an important mechanism in the observation that a lack of NQO1 activity affords an increased risk of benzene poisoning in exposed individuals [Rothman, N., et al. (1997) Cancer Res. 57, 2839-2842].

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (1) valid without restriction

21-JUL-2000 (1255)

**Type:** other: Environmental. Control of BTEX migration by intrinsic bioremediation at a gasoline spill site.

**Remark:** A full-scale, detailed intrinsic bioremediation investigation was conducted at a gasoline spill site in Dublin, North Carolina. Due to the appearance of non-aq. phase liq. (NAPL) hydrocarbons beneath the former spill location, dissolved BTEX (benzene, toluene, ethylbenzene, xylene) is continuously released from NAPL into the groundwater with a total BTEX concn. of 60 mg/L. At this spill site, a cropland extends from the mid-plume area to the down-gradient edge of the plume. Approx. 15 mg/L NO3--N was detected in groundwater beneath the cropland due to fertilizer use. During a 3-yr study, the following tasks were conducted: groundwater anal.; microbial enumeration; and mass flux and decay rate calcns. Results showed that BTEX concns. dropped to below detection limit (BDL) before they reached the down-gradient monitor well located 110 m from the spill location. Groundwater and microbial analyses

indicated Fe redn. was the dominant biodegrdn. process between the source and mid-plume area; however, a NO<sub>3</sub><sup>-</sup> spill in the cropland area switched the degrdn. pattern to denitrification, and changed the preferential removal of certain BTEX components. Under Fe-reducing conditions, toluene and o-xylene declined most rapidly followed by m- and p-xylene, benzene, and ethylbenzene. Within the denitrifying zone, toluene and m- and p-xylene degraded very rapidly, followed by ethylbenzene, o-xylene, and benzene. Mass flux calcns. showed that .ltoreq.93.1% of BTEX was removed within the Fe-reducing zone, and 5.6% of BTEX was degraded within the NO<sub>3</sub><sup>-</sup> spill zone. The remaining 1.3% was removed within the oxidized zone at the down-gradient edge of the plume. Toluene had the highest first-order decay rate (0.16%) detected in the Fe-reducing zone; benzene had the lowest rate (0.07%) within the denitrification area. Results showed that mixed intrinsic bioremediation processes (Fe redn., denitrification, methanogenesis, aerobic biodegrdn.) effectively contained the plume; Fe redn. played an important role in BTEX removal.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(598)

**Type:** other: High frequency of loss of heterozygosity at chromosome 11 in benzene-induced thymic lymphomas in p53<sup>+/-</sup> transgenic mice.

**Remark:** In an ongoing study, benzene inhalation exposure has induced a high frequency (>80%) of thymic lymphomas in p53<sup>+/-</sup> mice beginning at six months of exposure. To characterize the presence and extent of loss of heterozygosity (LOH) on chromosome 11, we have examined the status of seven microsatellite markers in 27 benzene-induced and six spontaneous thymic lymphomas. LOH at microsatellite markers is an indication of large genetic alterations such as gross deletions, recombination or chromosomal loss. Benzene-induced thymic lymphomas exhibited six patterns of LOH and 59% (16/27) of induced lymphomas exhibited the same pattern. Each of the six spontaneous thymic lymphomas analyzed showed a unique pattern of LOH, only one of which was exhibited by the benzene-induced thymic lymphomas. Over 90% (25/27) of the benzene-induced thymic lymphomas exhibited loss of the functional p53 allele, and the loss of p53 was accompanied by LOH at other microsatellite markers suggesting the LOH was a result of gross alteration of chromosome 11. The results indicate that benzene treatment is inducing a high frequency of LOH on chromosome 11 in p53<sup>+/-</sup> mice, and that loss of the functional p53 allele or some other gene on chromosome 11 is a key event in the induction of thymic lymphomas in these mice.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (4) not assignable

24-JUL-2000

(129)

**Type:** other: Cancer bioassay and genotoxicity of inhaled benzene in p53+/- and C57B1/6 mice.

**Remark:** P53+/- and wildtype C57B1/6 mice have been exposed to benzene by chronic inhalation to assess tumorigenic and genotoxic responses. To determine the time to maximum tumor induction, the length of exposure was for 36 weeks or until the survival in the benzene exposed groups reached 20% of unexposed controls. Mice were exposed to either 0 ppm or 3000 ppm x hr/week of benzene using three exposure regimens: 100 ppm (6 hrs/day 5 days/week); 100 ppm (10 hrs/day 3 days/week; MWF) or 200 ppm (5 hrs/day 3 days/week; MWF). The frequency of micronuclei in peripheral blood was determined at 1, 5, 13 and at 33-38 weeks of exposure. At 26 weeks of exposure to benzene the incidence of thymic lymphomas was: 22% in the 100 ppm exposure groups, 2% in the 200 ppm exposure group and 0% in the air controls. In the 100 ppm exposure groups, 36 weeks of benzene exposure induced a high frequency of thymic lymphomas in p53+/- mice, >80% incidence compared to 2% in unexposed controls. In the 200 ppm exposure group, the incidence of thymic lymphomas in p53+/- mice at 36 weeks was 17%. The incidence of thymic lymphomas in C57B1/6 mice at 36 weeks at all levels of benzene exposure was less than 5%. The frequency of micronuclei was elevated in all exposure groups at all time points examined. The increased frequency of micronuclei in peripheral blood was equivalent or greater in the C57B1/6 mice than in the p53+/- mice. These data indicate that benzene inhalation exposure is carcinogenic in p53+/- mice, but at these levels of benzene exposure p53+/- mice are not more sensitive to the genotoxic effects of benzene than wild type mice.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (4) not assignable

24-JUL-2000

(929)

**Type:** other: Environmental. EU risk assessment guidelines, Part III: Scenario analysis of a level III multimedia model using generic and regional data.

**Remark:** Regional PECs (Potential Environmental Concns.) calcd. with the software EUSES were compared with measured values using different emission and environmental distribution scenarios. The environmental data set recommended in EUSES (default data set) represents a generic std. region. In different scenarios the parameters of the generic region are replaced by concrete values, and estd. parameters (emissions, degrdn. rates and partition coeffs.) are substituted by measured or investigated values. Deviations with regard to the measured values can be up to three orders of magnitude. Despite the basically conservative approxns., underestimations can occur. However, these are usually due to poor monitoring data or inappropriate input values. The use of regional data instead of default parameters only slightly ameliorates the results. The use of real emission and degrdn. rates alone can improve the results significantly.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(96)

**Type:** other: Catalytic inhibition of topoisomerase II alpha by benzene metabolites.

**Remark:** Chronic exposure to benzene has been associated with hematotoxicity including the development of acute myelogenous leukemia (AML). Clonal cytogenetic aberrations are thought to play an important role in the pathogenesis of AML developing secondary to drug or chemical exposure. Recent studies have implicated the inhibition of topoisomerase II alpha, an essential nuclear enzyme that catalyzes the interconversion of various forms of DNA, as a mechanism to explain benzene-induced cytogenetic aberrations. While chemotherapeutic modalities that include topoisomerase poisons are also known to be leukemogenic, the factor determining the potential to produce cytogenetic abnormalities is dependent on the mechanism of inhibition (catalytic inhibition versus cleavable-complex stabilization). The purpose of this study was to determine the mechanism by which topoisomerase II alpha is inhibited by the following benzene metabolites: benzoquinone, 1,2,4-benzenetriol, 4,4-biphenol, and hydroquinone. In a DNA cleavage/relaxation assay where topoisomerase II alpha is exposed to different benzene metabolites prior to analysis of enzyme activity, we observe inhibition of topoisomerase II alpha with the above benzene metabolites at 30 microM, 100 microM, 100 microM, and 1.0 mM respectively. However, no stabilization of cleavable complex formation is formed and, in fact, an antagonistic effect on etoposide-stabilized cleavable complex formation is observed. Our data suggests that these benzene metabolites are actually catalytic inhibitors of topoisomerase II alpha and therefore are not likely to contribute to the development of AML through this mechanism.

**Source:** EXXON Biomedical Sciences East Millstone, NJ

**Reliability:** (4) not assignable

24-JUL-2000

(66)

**Type:** other: Environmental. Exposure to volatile organic compounds while commuting in Taichung, Taiwan.

**Remark:** The objective of this study was to compare volatile org. compds. (VOC) concns. from six main roads in Taiwan's third largest city (Taichung) and det. factors that affect VOC concns. Twenty-two VOC compds. were detd. on six roads using US EPA method TO-17. 0.2 gram of Carbopack B was used as an adsorbent collector and thermal desorption and gas chromatog./mass spectrometry (GC/MS) techniques were used to analyze VOCs. Results showed that car and motorcycle commuters were exposed to the highest VOC concns. on Ta-Ya Road (2149 and 1343 microgram/m<sup>3</sup>, resp.). Except for Ta-Ya Road and Chung-Kang Road, motorcycle commuters were exposed to higher VOC concns. than their driving counterparts. Four sampling periods were used in the study; exposure to VOC concns. was highest from 7:30 to 8:30 AM for motorcycle

commuters (1515 microgram/m<sup>3</sup>) and from 5:00 to 6:00 AM for car commuters (1071 microgram/m<sup>3</sup>), while concns. for all commuters were lowest from 2:00 to 3:00 P.M. Concns. were neg. correlated to percentage of vacant lots along the road (PVL), yet not correlated to traffic vol. The ratios for toluene, benzene, xylene, and ethylbenzene (T: B: X: E) were similar for car commuters (5.5: 2.1: 2.6: 1.0) and motorcycle commuters (5.7: 2.0: 2.6: 1.0) which, combined with other published data, strongly suggest a vehicular source for hydrocarbons in Taichung. Comparing these concns., Taichung was slightly lower than Taipei but 2-30 times higher than cities of other countries. It is concluded that the chronic exposure to high concns. of hydrocarbons may pose a serious health risk to commuters in Taichung.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(647)

**Type:** other: Environmental. Fate of benzene in a stratified lake receiving contaminated groundwater discharges from a Superfund site.

**Remark:** Predicting the fate of benzene in aquatic environments, and estg. corresponding human exposures, is critically dependent on knowledge of this carcinogen's biodegrdn. rate under the site-specific conditions. We used 3 approaches for quantifying this key fate process: (1) short-term (hours) observations of benzene loss in lab. incubations of representative water samples, (2) whole-lake benzene mass balance studies, and (3) modeling of the temporal evolution of benzene vertical profiles in the lake. Our field site, the Halls Brook Holding Area (HBHA), continuously receives benzene input (approx. 20 microM or 1.5 ppm) into its anoxic hypolimnion via discharge of saline groundwater from an adjacent Superfund site (Industri-Plex in Eastern Massachusetts). Using summertime lake water samples in the lab., we found benzene was degraded in 3 metalimnion samples at rates 1-2.5/day. An epilimnion sample yielded a similar result, but no degrdn. was obsd. in another epilimnion sample. Losses were 0.04/day in a sulfate-rich hypolimnion sample. Since benzene loss could be inhibited by filtration or with a mixt. of poisons and antibiotics, it was apparently being biodegraded. In the whole-lake mass balance studies of benzene, it was found that approx. 80% of the benzene entering the lake was degraded during the water's residence in the lake. Vertical distributions of benzene in the HBHA water column indicated that the chief sink of benzene was located in the metalimnion. A 2-mo progression of summertime profiles of benzene concn. vs. depth was fitted well using a dynamic model, CHEMSEE, and assuming that the only sinks were epilimnetic flushing, water-to-air exchange, and biodegrdn. in a 0.4 m-thick metalimnetic layer at 2/day. The biodegrdn. rate derived from such whole-system study appears more dependable than rates deduced from grab samples, and we suggest that we must learn to predict these intact-system rates.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
14-FEB-2002 (1254)

**Type:** other: Environmental. Future air quality in Danish cities : impact assessment of the new EU emission and fuel quality directives.

**Remark:** The impact of new EU vehicle emission and fuel quality directives on the future air quality in Danish cities has been modelled for comparison with new limit values in the new EU directive on assessment and management of urban air quality. The assessment is carried out for the reference year 1995 and the scenario years: 2000, 2005, 2010, 2015 and 2020 for selected streets in Copenhagen, Denmark. Modelled health related substances include: NO<sub>2</sub> (NO<sub>x</sub>), O<sub>3</sub>, CO and benzene. Preliminary assessment of particulate matter is based on expert judgement. Nested modelling was applied using a set of air quality and emission models to predict concentration levels in the regional background, urban background and in the street. Air pollution levels were predicted to decrease for all pollutants except for ozone and the results show that the levels will not exceed the new EU limit values in 2010 despite expected increases in traffic.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
14-FEB-2002 (581)

**Type:** other: Environmental. Natural biological attenuation of benzene in groundwater.

**Remark:** Benzene was found in subsurface unsatd. soil and groundwater beneath a petro-chem. plant (China). Although the groundwater contained several mg/L of benzene in the area immediately beneath the source, benzene was not detected in monitoring wells approx. 800 m down stream. All kinds of phys. processes such as adsorption and advection/dispersion are considered to account for the obsd. attenuation. The attenuation was primarily due to natural biol. processes occurring within the aquifer. The evidence for the natural bioremediation of benzene from the groundwater included: (1) anal. of groundwater chem., (2) lab. studies demonstrating benzene biodegrdn. in aquifer samples, and (3) computer simulations examg. benzene transport. Lab. expts. indicated that for conditions similar to those in the plume, the aerobic degrdn. of benzene by the naturally occurring microorganisms in the polluted groundwater samples was quite rapid with a half-life time of from 5-15 days. In situ analyses indicated the level of dissolved O in the groundwater was over 2 mg/L. Thus, O should not limit the biodegrdn. In fact, the benzene was also shown to degrade under anaerobic conditions. Biodegrdn. is the dominant process influencing attenuation of the benzene.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
14-FEB-2002 (697)

**Type:** other: Environmental. Physical versus biological hydrocarbon removal during air sparging and soil vapor extraction.

**Remark:** Although physical removal of contaminants may account for the majority of mass of petroleum hydrocarbons (PH) removed using air sparging and soil vapor extraction (AS/SVE), biological processes contribute to mass removal both during system operation and during system shutdown. Biodegradation rates are difficult to measure during active AS/SVE due to the forced entry of air. This study examined the relative rates of physical and biological removal of PH during AS/SVE. Before system startup, soil gas CO<sub>2</sub> and O<sub>2</sub> were measured to estimate biodegradation rates based stoichiometrically on the mass of hexane. Biodegradation rates calculated from average CO<sub>2</sub> production (0.08%/d) were 1.3 times less than those based on average O<sub>2</sub> utilization (0.26%/d). A simulation model (Stella II) incorporating air injection and microbial respiration measured during AS/SVE shutdown predicted initial O<sub>2</sub> concentrations during AS/SVE well but overestimated steady-state O<sub>2</sub> by up to 2.5% potentially because respiration may be underestimated if more O<sub>2</sub> depletion occurred during AS/SVE than was measured when the system was off. Although biodegradation accounted for 77 times less contaminant mass removal than physical removal, biological contaminant removal may be underestimated using standard respiration tests.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(12)

**Type:** other: Environmental. The use of environmental risk assessment methodologies for an indoor air quality investigation.

**Remark:** The authors of this paper chose several target compds. that have been found in av. US homes, applied the current US Environmental Protection Agency (US EPA) Superfund risk assessment methods. to indoor air quality, and produced risk numbers. for hazard quotients and predicted increases in incidence of cancer which would be unacceptable at US hazardous waste sites. The calcns. were made for the av. child and adult with US EPA default exposure values. Calcns. were also made for a worst-case scenario using max. concns. and exposure ests. defined by the US EPA as describing the reasonable exposure (RME). Significant cancer risks and non-cancer hazard quotients were predicted.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey

14-FEB-2002

(511)

**Type:** other: Environmental. Urban benzene and population exposure.

**Remark:** Urban populations are exposed to both indoor and outdoor benzene pollution in Europe. The indoor air benzene concn. tends to be higher than the outdoor concn.; and the ratio is higher in the cities of northern Europe than in the southern.

**Source:** ExxonMobil Biomedical Sciences Inc. Annadale, New Jersey  
14-FEB-2002 (220)

**Type:** adsorption

**Remark:** method: percutaneous absorption of <sup>14</sup>C-labelled benzene in male hairless mice was measured, following 4 h administration of a) undiluted benzene or b) 0.5% benzene in rubber solvent using special skin-depot attachment. results: a) 0.89%, b) 0.88% absorbed. a) 33% of absorbed dose excreted (urine, faeces), 40% expired, 23% in carcass and 5% at application site; similar results in b). Estimation by the authors: dermal absorption could contribute 20-40% of the total benzene dose of workers in rubber industry (1 ppm benzene in air).  
Reliability: 1  
Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn, German rapporteur  
**Flag:** Risk Assessment  
14-SEP-2000 (1100)

**Type:** adsorption

**Remark:** The pathways for benzene oxidative metabolism are generally understood and involve the cytochrome P-450 family of enzymes. Transgenic CYP2E1 knockout mice were used to investigate the involvement of CYP2E1 in the in vivo metabolism of benzene and in the development of benzene-induced toxicity. For the metabolism studies, male transgenic and wild-type control mice were exposed to 200 ppm benzene, along with a radiolabeled tracer dose <sup>14</sup>C-benzene by nose-only inhalation for 6 hr. Total urinary radioactivity and all radiolabeled individual metabolites were reduced in urine of transgenic mice compared to wild-type controls during the 48-hr period after benzene exposure. In addition, a significant greater percentage of total urinary radioactivity could be accounted for as phenylsulfate conjugates in transgenic mice compared to wild-type mice, indicating the importance of Cyp2E1 in oxidation of phenol following benzene exposure in normal mice. Because benzene oxidative metabolism was not completely abolished in transgenic CYP2E1 knockout mice, metabolism must occur through other metabolic enzymes. Hepatic CYP2B1 metabolizes benzene in rat liver microsomes, although at much higher concentrations than those needed for catalysis by CYP2E1. It is likely that this enzyme has a significant contribution in the oxidative metabolism of benzene in vivo in transgenic CYP2E1 knockout mice. For the toxicity studies, male transgenic CYP2E1 knockout, wild-type, and B6C3F1 mice were exposed by whole-body inhalation to 0 ppm or 200 ppm benzene, 6 hr/day for 5 days. On day 5, blood, bone marrow, thymus, and spleen were removed for evaluation of micronuclei frequencies and tissue cellularities. No benzene-induced cytotoxicity or genotoxicity was observed in transgenic CYP2E1 knockout

mice. In contrast, benzene exposure resulted in severe genotoxicity and cytotoxicity in both wild-type and B6C3F1 mice. These studies conclusively demonstrate that CYP2E1 is the major determinant of in vivo benzene metabolism and benzene-induced myelotoxicity in mice.

**Source:** German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000 (1198)

**Type:** Behaviour

**Remark:** method: groups of 8 male Sprague-Dawley rats received 3 i.p. injections of 550 mg/kg/d benzene in corn oil or the vehicle on day 9, 11, 13 postpartum.  
results: no effects in body weight gain, food and water consumption; significantly impaired learning ability when tested on problems of the Hebb-Williams closed-field maze-learning task on day  $\geq$  54.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** purity >99%

06-JAN-1997 (398)

**Type:** Biochemical or cellular interactions

**Remark:** Inhaled  $^3\text{H}$ - or  $^{14}\text{C}$ -labelled benzene had been found to bind to adult male SIV50 rat liver DNA to the extent of 2.40 or 2.38 umoles benzene per mole DNA phosphate. Seven deoxyguanosine adducts and one deoxyadenine adduct have been detected in rabbit bone marrow mitoblast DNA (incubation 1st. with  $^3\text{H}$ -labelled dGTP or dATP, than with  $^{14}\text{C}$ -benzene).

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997 (713) (1075)

**Type:** Biochemical or cellular interactions

**Remark:** The hemoglobin adduct, S-phenylcysteine, was detected in the blood of mice and rats exposed to benzene either by inhalation or by gavage. Both covalently bound and soluble metabolites accumulated in bone marrow, liver, and kidney over a 24-h-period after a single s.c. administration of  $^3\text{H}$ -labelled benzene (440 or 880 mg/kg) to male mice; the highest level of covalent binding were seen in kidney and liver after 3 d of dosing at 880 mg/kg, 2 doses per day.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997 (88) (701)

**Type:** Biochemical or cellular interactions

**Remark:** A metabolite of benzene, Hydroquinone (HQ) is further metabolized to p-benzoquinone (BQ) in a peroxidase-mediated reaction in myeloid progenitor cells. The ability of the compounds to inhibit the activity of topoisomerase II (topoII) was tested using an assay system that depends on the conversion, by homogeneous human topo II, of catenated kinetoplast DNA into open and/or nicked open circular DNA that can be separated from the catenated DNA by electrophoresis. Both HQ and BQ cause a time and concentration ( $\mu\text{M}$ )-dependent inhibition of topo II activity. BQ does not stimulate the production of linear DNA indicative of an inhibition of topo II religation of strand breaks by stabilization of the covalent topo II-DNA cleavage complex. Rather, BQ most probably inhibits the SH-dependent topo II by binding to an essential SH group. The inhibition of topo II by BQ has implications for the formation of deleterious translocations that may be involved in BZ-induced initiation of leukemogenesis.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(546)

**Type:** Distribution

**Remark:** Rats were exposed to 500ppm benzene for 30 min to 8h. Benzene concentrations reached steady state within 4h in blood, 6h in fat and 2h in bone marrow. Lesser concn. were detected in the kidney, lung, liver, brain and spleen.

**Source:** REPSOL PETROLEO, S.A. MADRID; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(935)

**Type:** Distribution

**Remark:** method: 4 pregnant C57BL mice per group were exposed to 2000 ppm (6.4 mg/l) benzene for 10 min.; mice were sacrificed 0, 0.5, 1, 4, and 24 h after inhalation for whole-body autoradiography and liquid scintillation methods.  
results: immediately after inhalation high concentration in lipid-rich tissue (brain, fat) and well perfused organs (lung, liver, kidney) but rapidly eliminated resulting in low concentrations at 1 h in all maternal tissues, except fat; volatile radioactivity was observed in placenta and fetuses at 0, 0.5, and 1 h after inhalation; fetal levels much lower than in maternal tissue.

Reliability: 1

Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(407)

**Type:** Distribution

**Remark:** Gas chromatographic-mass spectrometric analysis of profiles of low molecular weight volatile organic constituents obtained from cord blood and maternal blood samples collected at birth reflect transplacentally acquired compounds. In the 11 paired cord blood-maternal blood samples analyzed, the relative amounts of constituents in cord blood closely correspond to those quantities present in the maternal blood. Benzene, is present in cord blood in quantities equal to or greater than in maternal blood.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(308)

**Type:** Immunotoxicity

**Remark:** Immunoglobulin levels in the sera of 35 workers occupationally exposed to benzene, toluene and xylene were studied by means of a simple radial immunodiffusion. The immunoglobulin levels were compared with those of a control group of 42 healthy adults. A statistically significant decrease was observed in the IgG and IgA levels, accompanied by an increase in the IgM level. It is possible that this observation reflects a suppressive action of benzene on immunoglobulin-producing cells, resulting in the inhibition of DNA synthesis.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(661)

**Type:** Immunotoxicity

**Remark:** Pretreatment of Ficoll-purified rat spleen lymphocytes with hydroquinone (HQ) enhanced in vitro phytohemagglutinin (PHA)-stimulated mitogenesis at lower concentrations but inhibited lectin-stimulated blast transformation at higher concentrations. Following preincubation with HQ, viable lymphocytes failed to agglutinate or undergo blast transformation in the presence of PHA. This effect occurred in the absence of depressed energy production which suggested an impairment of cytoskeletal function. Other polyhydroxy metabolites of benzene, including p-benzoquinone, 1,2,4-benzenetriol, and catechol produced similar effects on mitogen response. N-Ethylmaleimide (NEM), a membrane penetrating alkylating agent with specificity for sulfurhydryl (SH) groups, inhibited lymphocyte mitogenesis and agglutination at the same concentrations as HQ. 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB), a poorly penetrating SH reagent, had no effect on lymphocyte function. The concurrent inhibition of mitogenesis and agglutination by HQ and NEM was prevented by the addition of dithiothreitol (DTT), a SH compound, suggesting an important interaction of the metabolite (or its products) with intracellular SH groups critical to early events in blastogenesis. The interaction of polyhydroxy

metabolites of benzene with particularly reactive SH groups on microtubules may explain their sublethal effects on lymphocyte function.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(888)

**Type:** Immunotoxicity

**Remark:** Preincubation with > 1 uM hydroquinone (HQ) suppressed blastogenesis of rat splenic lymphocytes. p-Benzoquinone is approximately twice as effective as HQ in suppressing blastogenesis of lymphocytes. Catechol and 1,2,4-benzenetriol are also effective. Dithriothreitol protects against the inhibitory effect of HQ suggesting a role for sulfhydryl groups. Both HQ and p-benzoquinone (BQ) inhibited formation of microtubules in vitro. The sulfhydryl groups on tubulin may be uniquely sensitive to quinone metabolites of immunotoxic chemicals.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(889)

**Type:** Immunotoxicity

**Remark:** The studies in this report involved the interaction of inhaled benzene with some of those cell-mediated immune responses associated with tumor surveillance. Exposures to 100 ppm benzene (5 days/week x 20 weeks) induced lethal tumor growth in 9/10 C57B1/6 mice inoculated with 10(4) viable PYB6 tumor cells. Lethal tumor incidences in air controls and mice exposed to lower benzene concentrations were 3/10 or less. Exposures to 100 ppm benzene (5 days/week x 4 weeks) also reduced the tumor lytic abilities of cytotoxic T lymphocytes as determined by 51Cr-release assays. In addition, splenocytes taken from mice exposed to 10 or 100 ppm benzene (5 days/week x 4 weeks) exhibited delays in peak mixed leukocyte responses. Coculture experiments demonstrated that these delays were not due to an induction of suppressor cell activity by benzene. There were no alterations in the relative percentages of B cells, T cells, or T-cell subsets among splenocytes from animals exposed to any concentration of benzene tested. These results demonstrate that inhaled benzene can inhibit some of the processes associated with tumor surveillance, and that this inhibition is due, at least in part, to impairments of the functional abilities of some of the cells responsible for tumor surveillance.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(957)

**Type:** Immunotoxicity

**Remark:** Benzene inhibits the maturation of early blood cell precursors. DNA synthesis is reduced in bone marrow of benzene-treated animals. Acute leukemia is frequently related to benzene exposure. Specific immunoglobulins such as IgA and IgG as well as serum complement are depressed while IgM is elevated in benzene intoxication. Benzene-intoxicated individuals often suffer from serious infections which they are unable to combat and thus may be terminal. Furthermore, in some instances of benzene toxicity the "immune surveillance" mechanism which appears to be partly responsible for preventing the growth and development of neoplastic tissues may not function and the result may be benzene-induced leukemia.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1074)

**Type:** Metabolism

**Remark:** Benzene is well absorbed in humans and experimental animals after oral and inhalation exposures, but in humans dermal absorption is poor. Approximately 50% absorption occurs in humans during continuous exposures to 163-326 mg/m<sup>3</sup> for several hours. After a 4-hr exposure to 170-202 mg/m<sup>3</sup>, retention in the human body was approximately 30%, with 16% of the retained dose having been excreted as unchanged benzene in expired air. Women may retain a greater percentage of inhaled benzene than men. Benzene tends to accumulate in tissues containing high amounts of lipids, and it crosses the placenta.

Benzene metabolism occurs mainly in the liver, is mediated primarily through the cytochrome P-450 IIE1 enzyme system and involves the formation of a series of unstable reactive metabolites. In rodents, the formation of two putative toxic metabolites, benzoquinone and muconaldehyde, appears to be saturable. This may have important implications for dose-response relationships, because a higher proportion of the benzene will be converted to toxic metabolites at low doses than at high doses. The metabolic products are excreted primarily in the urine. Appreciable levels of the known metabolites phenol, catechol and hydroquinone are found in bone marrow. Phenol is the predominant urinary metabolite in humans and is mainly found as an ethereal sulphate conjugate until the levels approach 480 mg/l, at which time glucuronides are detected. Recent studies suggest that benzene toxicity is the result of the interactive effects of several benzene metabolites formed in both the liver and the bone marrow.

Inhaled benzene had been found to bind to rat liver DNA to the extent of 2.38  $\mu$ moles/mole DNA phosphate. Seven deoxyguanosine adducts and one deoxyadenine adduct have been detected in rabbit bone marrow mitochondrial DNA.

**Source:** BP Chemicals Ltd LONDON

13-DEC-1996

(1252)

**Type:** Metabolism**Remark:** Metabolic products in rat are phenol, hydroquinone, catechol, hydroxyhydroquinone and phenylmercapturic acid.**Source:** REPSOL PETROLEO, S.A. MADRID

13-DEC-1996

(813)

**Type:** Metabolism

**Remark:** Reliability: 4 (validity cannot be judged)  
data from secondary literature, but validated (WHO, 1993) by an expert group (WHO task group)  
The major metabolism of benzene in vivo takes place in liver and, to lesser extent, in bone marrow; first step is oxidative, yielding hydroxylated compounds (phenol, catechol, hydroquinone, 1,2,4-trihydroxy-benzene) which are excreted in the urine as ethereal sulfates and glucuronides; Conjugation with glutathione and urinary excretion of the mercapturic acid is an additional pathway that detoxifies benzene oxide (initial intermediate formed during metabolism to phenol); urinary metabolite muconic acid is the only known ring-opened metabolite, a detoxification product of trans,trans-muconaldehyde; further metabolism of the metabolite phenol lead to formation of hydroquinone, catechol and 1,2,4-trihydroxy benzene; further metabolism of catechol leads to o-benzoquinone and hydroquinone leads to p-benzoquinone.  
In summary, benzene metabolism occurs mainly in the liver, is mediated primarily through the cytochrome P-450 IIE1 enzyme system and involves the formation of a series of unstable reactive metabolites. In rodents, the formation of two putative toxic metabolites, benzoquinone and muconaldehyde, appears to be saturable. This may have important implications for dose-response relationships, because a higher proportion of the benzene will be converted to toxic metabolites at low doses than at high doses. The metabolic products are excreted primarily in the urine. Appreciable levels of the known metabolites phenol, catechol and hydroquinone are found in bone marrow. Phenol is the predominant urinary metabolite in humans and is mainly found as an ethereal sulphate conjugate until the levels approach 480 mg/l, at which time glucuronides are detected. Recent studies suggest that benzene toxicity is the result of the interactive effects of several benzene metabolites formed in both the liver and the bone marrow.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur**Flag:** Risk Assessment

14-SEP-2000

(874) (1076) (1252)

**Type:** Metabolism

**Remark:** method: a) male F344/N rats or male B6C3F1 mice inhaled 50 ppm 3H-labelled benzene for 6 h and 0.5, 2, 4, 6, 7.5, 9, 11, 14 h after start of exposure 4 animals of each species were sacrificed and metabolites determined; b) both species received i.p. injections of 0.5, 5, 15, 50, 150 mg/kg benzene or inhaled 11, 130, 930 ppm for 6 h and metabolites were determined during a 48-h-period after dosing.  
results: a) in rats the metabolites (measured as areas under the curve in liver, lung and blood over the 14 h period) were mainly phenyl sulfate, muconic acid, phenyl mercapturic acid and in small quantities phenol, catechol, hydroquinone; in mice the main metabolites were muconic acid, phenyl sulfate, phenyl mercapturic acid, hydroquinone glucuronide, phenyl glucuronide, hydroquinone and small amounts catechol and phenol; mice converted more inhaled benzene to putative toxic metabolites, benzoquinone and muconaldehyde, than rats did. b) the effect of increasing doses was to increase the proportion of the total metabolites that were products of detoxification pathways relative to the products of pathways leading to putative toxic metabolites in both species. This indicates high-affinity, low-capacity pathways leading to putative toxic metabolites.

Reliability: 1 (valid)

Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(499) (762) (987) (988)

**Type:** Metabolism

**Remark:** Monoclonal antibody-directed characterization of benzene oxidative metabolism in rat liver microsomes showed that benzene is a substrate for the cytochrome P450IIE1 and at higher benzene concentrations for P450IIB.

Reliability: 1

Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(808)

**Type:** Metabolism

**Remark:** Bone marrow, bone marrow-derived macrophages, and stromal fibroblasts were isolated from male B6C3F1 mice in order to more completely characterize the enzymology contributing to the metabolic activation and deactivation in bone marrow-derived macrophages and fibroblastoid stromal cells. The investigators examined the levels of glutathione (GSH) and the activities of phase II metabolic pathways that are critical in the deactivation of benzene-derived phenolic metabolites and quinones formed by further oxidation of these phenolics. Specifically, the activities of UDP-glucuronosyltransferase (UDP-GT); (EC 2.4.1.17), PAPS-dependent arylsulfotransferase (EC.2.8.2.1), and

glutathione-S-transferase (EC 2.5.1.18) were examined in unpurified white bone marrow, bone marrow-derived macrophages, and stromal fibroblasts. Fibroblastoid stromal cells had elevated glutathione-S-transferase and DT-diaphorase activity relative to macrophages, whereas macrophages demonstrated increased UDP-GT and peroxidase activity relative to stromal fibroblasts. UDP-GT and glutathione-S-transferase activities in macrophages and fibroblasts, respectively, were significantly greater than those in unpurified white marrow. Because UDP-GT activity is high in macrophages, these data suggest DT-diaphorase levels would be rate limiting in the detoxification of benzene-derived quinones in bone marrow macrophages. The peroxidase responsible for bioactivation of benzene-derived phenolic metabolites in bone marrow macrophages is unknown but has been suggested to be prostaglandin H synthase (PGS). Hydrogen peroxide, but not arachidonic acid, supported metabolism of hydroquinone to reactive species in bone marrow-derived macrophage lysates. These data do not support a major role for PGS in peroxidase-mediated bioactivation of hydroquinone in bone marrow-derived macrophages. Peroxidase-mediated interactions between phenolic metabolites of benzene occurred in bone marrow-derived macrophages. Bioactivation of hydroquinone to species that would bind to acid-insoluble cellular macromolecules was increased by phenol and was markedly stimulated by catechol. Bioactivation of catechol was also stimulated by phenol, but was inhibited by hydroquinone. These data define the enzymology and the cell-specific metabolism of benzene metabolites in bone marrow stroma and demonstrate that interactions between phenolic metabolites may contribute to the toxicity of benzene in this critical bone marrow compartment.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(392)

**Type:** Metabolism

**Remark:** This study investigates the prostaglandin H synthase (PHS) catalyzed oxidation of the benzene metabolite hydroquinone and examines the sulfhydryl-binding and DNA-damaging effects of the reactive metabolite generated during this reaction. The study also compares the activation of hydroquinone by PHS to those of other purified peroxidase systems. Hydroquinone was oxidized by PHS to 1,4-benzoquinone, which was measured by HPLC with reductive electrochemistry. Hydroquinone metabolism in the presence of cysteine generated a thiol adduct, which was identified as the monosubstituted cysteine conjugate of hydroquinone by HPLC with oxidative electrochemical and radiochemical detection. The PHS-catalyzed activation of hydroquinone to 1,4-benzoquinone or its thiol conjugate required the presence of either arachidonic acid or hydrogen peroxide. The oxidative metabolism of hydroquinone also resulted in the formation of a reactive product(s) that irreversibly bound the DNA. This binding was time dependent and did not

occur in the presence of heat-inactivated PHS. Metabolite(s) generated during hydroquinone oxidation also induced single-strand breaks in Bluescript plasmid DNA. The PHS/arachidonic acid catalyzed metabolism of hydroquinone to 1,4-benzoquinone and to product(s) that bound to sulfhydryls and DNA and caused strand breaks in DNA was prevented by indomethacin, an inhibitor of PHS cyclooxygenase. Because prostaglandin synthesis is elevated in bone marrow following benzene exposure and inhibitors of PHS cyclooxygenase prevent benzene-induced myelotoxicity, the activation of hydroquinone by PHS represents a possible mechanism for benzene's effects.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1014)

**Type:** Metabolism

**Remark:** In this study, benzene metabolism was investigated using two purified rat hepatic mixed function oxidase (MFO) systems containing either cytochrome P450 2B1 or cytochrome P450 2E1. Studies performed over a wide substrate concentration range indicate that cytochrome P450 2B1 represents a relatively low-affinity form of cytochrome P450 with respect to benzene metabolism while cytochrome P450 2E1 is substantially more efficient at low benzene concentrations. Cytochrome b5 stimulated benzene metabolism by both cytochromes P450 2B1 and P450 2E1. With cytochrome P450 2E1 the stimulation of benzene metabolism by cytochrome b5 was very pronounced at low concentrations of benzene and was most effective with respect to formation of hydroquinone. The metabolites observed in these studies were phenol and hydroquinone. Cytochrome P450 2E1 metabolized phenol with an affinity and capacity comparable to those of benzene. Hydroquinone was the major product formed at all substrate concentrations, while some catechol was formed at higher concentrations of phenol. Phenol metabolism was also stimulated by cytochrome b5. The metabolism of benzene by cytochrome P450 2E1 in the presence of the major microsomal epoxide hydrolase, mEHb yielded phenol, hydroquinone, and benzene dihydrodiol. The addition of glutathione transferases plus glutathione (GSH) did not yield GSH conjugates during benzene metabolism. However, metabolism of phenol by cytochrome P450 2E1 in the presence of glutathione yielded a nonenzymatically formed glutathione conjugate derived from hydroquinone or from an oxidative product of hydroquinone. Studies of the metabolic pathway of benzene and its control are important as they may demonstrate that the development of benzene toxicity can be altered by changing the rate of production of mixtures of toxic metabolites.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Flag:** Risk Assessment

06-JAN-1997

(1068)

**Type:** Metabolism

**Remark:** Benzene metabolism is a requirement for toxicity and the phenolic metabolites of benzene have been repeatedly implicated in hematotoxicity. Peroxidase-mediated metabolism of benzene-derived phenolic compounds generates reactive and cytotoxic quinones which have been proposed to be responsible for benzene-induced toxicity. Human as well as animal (i.e. rat and mouse) bone marrow contains high peroxidase activity and can readily bioactivate phenolic metabolites of benzene in a peroxidase-mediated process. Thus, bone marrow cells containing significant peroxidase activity can be considered as potential targets of benzene toxicity. In this study, the authors examined the peroxidase activity of murine hematopoietic progenitor cells (HPC) by two different approaches. Conventional biochemical techniques were used to quantitatively determine peroxidase activity in enriched progenitor cell populations, purified as lineage-negative cells using a cocktail of antibodies to remove cells with lineage-specific markers. Peroxidase activity in enriched progenitor cells and in whole bone marrow was also measured directly using flow cytometry. In addition, the peroxidase activity of HPC defined as CD34-enriched populations was also measured. The data demonstrate that both murine and human HPC contain peroxidase activity and thus may be potential targets of xenobiotics that are bioactivated by peroxidases.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1011)

**Type:** Metabolism

**Remark:** The administration of [3H]benzene by S.C. injection to mice resulted in the decreased incorporation of Fe into red cells and the accumulation of benzene and its metabolites in bone marrow and other tissues. Toluene protected against the benzene-induced depression of red cell Fe uptake and reduced the levels of benzene metabolites in bone marrow without affecting the level of benzene in this tissue. The results of this study suggest that toluene exerted its protective effect by inhibiting benzene metabolism and that a metabolite of benzene probably mediates the observed hemopoietic toxicity of benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(33)

**Type:** Metabolism

**Remark:** Metabolite information is used to construct an internal dose (a surrogate of the biologically effective dose) for a given administered dose. The relationship between the administered dose and this internal dose is nonlinear and is well described by a Michaelis-Menten function. The administered doses from the National Toxicology Program's rodent carcinogenicity study of benzene are transformed

into internal doses, and these internal doses are used in conjunction with a multistage model to compare previous estimated virtually safe doses (VSD) associated with small added health risks. The ratio of VSD for the administered dose risk assessment to the VSD from the internal dose risk assessment was approximately 1.0 for the F344/N rats and ranged from 2.5 to 5.0 for B6C3F1 mice in the National Toxicology Program study. For an occupational exposure of 1ppm, a risk estimate of 0.7 excess cancers/1000 exposed with an upper bound 3.5/1000 was obtained for a total metabolite internal dose risk assessment.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(65)

**Type:** Metabolism

**Remark:** Cancer risk from exposure to benzene for a working lifetime was estimated from data obtained in studies with rodents. Cancers of the Zymbal gland and the blood-forming system were selected as endpoints for the assessment because of their consistent occurrence. The combined metabolites were judged from toxicological data to be the best representative of the reactive agent. Because of similarity in the percentages of lifetime exposed in the rodent studies and in the occupational setting, the amount metabolized/day as a result of exposures 5 days a week for a lifetime was judged to be an appropriate dose paradigm for this assessment. Derived Michaelis-Menton constants were used to convert the doses of combined metabolites from the pharmacokinetic studies to the doses used in the bioassays. Scaling across species was based on allometric relationships. Experimental data were used to scale doses across species with body weight ratios raised to the exponents of 0.74 for the inhalation route and 1.0 for the oral route. The occupational lifetime cancer risk estimated from rodent data was 6 to 14 cases/1000 workers, which is consistent with the 9.5 to 174 leukemia cases/1000 estimated by others from epidemiological data.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(91)

**Type:** Metabolism

**Remark:** A physiological pharmacokinetic model for benzene, incorporating metabolic transformations, is used to explore why benzene, but not phenol is carcinogenic at many sites in rats. The model has been parametrized using in vitro or in vivo experimental data. Ranges, rather than fixed values were assigned to the parameters. The model-predicted level of phenol and hydroquinone in the tissue are consistently higher when phenol, rather than benzene, is administered. This result demonstrates that the differential carcinogenicity of the two compounds is not explainable in the context of this pharmacokinetic analysis.

- It also indicates that the phenol hydroquinone pathway alone is unlikely to account for the carcinogenic effects of benzene. Other metabolites must therefore also be involved.
- Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment
- 14-SEP-2000 (128)
- Type:** Metabolism
- Remark:** Following the co-administration of phenol and hydroquinone, a synergistic increase in myelotoxicity and genotoxicity was observed in the bone marrow of mice. Micronuclei (MN) formed in bone marrow erythrocytes following the co-administration of these two metabolites was studied. A marked increase in MN was observed in mice co-administered phenol and hydroquinone, which was significantly greater than that observed with the individual metabolites. The major increase in MN induced by the phenol and hydroquinone combination originated from breakage in the euchromatic region of the mouse chromosomes. The origin of MN in mice co-administered phenol and hydroquinone differed substantially from that induced by hydroquinone alone, but was almost identical to that seen in MN from benzene-treated mice.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (191)
- Type:** Metabolism
- Remark:** The inhibitory effects of various phenolic and quinone metabolites of benzene on the activity of human topoisomerases I and II were studied in vitro. No inhibition of topoisomerase I was seen with any of the tested metabolites. Inhibitory effects on topoisomerase II were not observed for hydroquinone, phenol, 2,2'-biphenol, 4,4'-biphenol and catechol at concentrations as high as 500 uM. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited topoisomerase II at relatively high 500 and 250 uM concentrations, respectively. However, following bioactivation using a peroxidase/H<sub>2</sub>O<sub>2</sub> system, inhibitory effects were seen at concentrations as low as 50 uM for both phenol and 2,2'-biphenol and 10 uM for 4,4'-biphenol. The addition of reduced glutathione (GSH) to the 4,4'-biphenol and horseradish peroxidase reaction system protected topoisomerase II from inhibition suggesting that diphenoquinone or another oxidation product formed from 4,4'-biphenol might be the reactive species. These in vitro results indicate that inhibition of topoisomerase II may contribute to the clastogenic and carcinogenic effects of benzene.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (192)

**Type:** Metabolism

**Remark:** Work was performed using recently developed molecular cytogenetic techniques to investigate the aneuploidy-inducing and clastogenic properties of the major quinone-forming metabolites of benzene. The metabolites of benzene (hydroquinone, catechol, and benzenetriol) were shown to be capable of interfering with chromosome segregation and inducing chromosomal breakage. These results indicate that both numerical and structural chromosomal aberrations induced by the quinone metabolites of benzene may play a role in the carcinogenic effects of benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(317)

**Type:** Metabolism

**Remark:** Benzene-induced myelotoxicity was reproduced by the coadministration of two principal metabolites, phenol and hydroquinone. Coadministration of phenol (75 mg/kg) and hydroquinone (25-75 mg/kg) twice daily to B6C3F1 mice for 12 days resulted in a significant loss in bone marrow cellularity in a manner exhibiting a dose-response. Addition of phenol to incubations containing horseradish peroxidase, H<sub>2</sub>O<sub>2</sub>, and hydroquinone resulted in a stimulation of both hydroquinone removal and benzoquinone formation. Phenol-induced stimulation of hydroquinone metabolism and benzoquinone formation represents a likely explanation for the bone marrow suppression associated with benzene toxicity.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(318)

**Type:** Metabolism

**Remark:** Mouse liver microsomes metabolized benzene more rapidly than microsomes prepared from rat and rabbit liver. Treatment of mice with benzene increased the metabolism of benzene in vitro without increasing cytochrome P-450 concentrations. Conversely, treatment of mice with phenobarbital increased cytochrome P-450 values but did not increase benzene metabolism. Benzene metabolism was inhibited by compounds known to interact with the mixed function oxidase system, e.g., aniline, metyrapone, aminopyrine, SKF-525A and cytochrome c, but not by KCN or 3-amino-1,2,4-triazole. The evidence suggests that benzene metabolism is mediated by the mixed function oxidase and binding of benzene to cytochrome P-450 is a significant factor in determining the rate of benzene metabolism.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(428)

**Type:** Metabolism

**Remark:** Metabolism and toxicokinetic studies indicate the importance of determining both the disposition of the parent compound and its metabolites as well. Benzene metabolism leading to the formation of putative toxic metabolites including muconaldehyde and benzoquinone is by a high affinity, low-capacity process. However, detoxification by phenyl conjugates is by a low-affinity, high-capacity pathway. Therefore, at the high dose levels utilized in rodent toxicity studies, a shift in the metabolism of benzene toward detoxification pathways occurs. In order to model disposition of benzene and its metabolites in humans, some information on human metabolism of benzene is required, with this information, appropriate adjustment to the physiological parameters of the model can be made.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(498)

**Type:** Metabolism

**Remark:** Quantitatively, the liver is the major site of benzene metabolism in the body. Phenol, catechol and hydroquinone are found in rat blood and bone marrow following inhalation of benzene. However, in this study the concentrations of benzene metabolites in the bone marrow is much higher due to high retention. The metabolite concentration ratio between rat bone marrow and blood approached 400-fold.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(572)

**Type:** Metabolism

**Remark:** On incubation with rabbit liver microsomes, benzene oxide is converted enzymatically to trans-1,2-dihydro-1,2-dihydroxybenzene. The enzyme, "epoxide hydrolase," is found in both the microsomal and the soluble fractions and converts a variety of epoxides to 1,2-glycols. The trans-1,2-dihydro-1,2-dihydroxybenzene is dehydrogenated to catechol by the supernatant of microsomes. In the presence of acid, benzene oxide readily rearranges to phenol. This isomerization is also catalyzed by proteins, simple peptides, and acetamide. Consequently phenol is a major nonenzymatic product in all enzymatic studies with benzene oxide. An enzyme in the soluble fraction of rat liver catalyzes the addition of glutathione to benzene oxide forming the premercapturic acid, S-(1,2-dihydro-2-hydroxyphenyl)-glutathione.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(582)

**Type:** Metabolism

**Remark:** Benzene is metabolized by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow. Bone marrow contains high levels of myeloperoxidase which can catalyze the further metabolism of the phenolic metabolites to reactive free radical species. Redox cycling of these free radical species produces active oxygen. This active oxygen may damage cellular DNA (known as oxidative DNA damage) and induce genotoxic effects. HL60 cells (a human leukemia cell line) contain high levels of myeloperoxidase and were used as an in vitro model system. Exposure of these cells to phenol, hydroquinone, and 1,2,4-benzenetriol resulted in an increased level of oxidative DNA damage. An increase in oxidative DNA damage was also observed in the mouse bone marrow in vivo 1 h after benzene administration. A dose of 200 mg/kg benzene produced a 5-fold increase in the 8-hydroxydeoxyguanosine level. Combinations of phenol, catechol, and hydroquinone also resulted in significant increases in steady state levels of oxidative DNA damage in the mouse bone marrow but were not effective when administered individually. Administration of 1,2,4-benzenetriol alone did, however, result in a significant increase in oxidative DNA damage. This represents the first direct demonstration of active oxygen production by benzene to phenolic metabolites and the subsequent production of oxidative DNA damage may therefore play a role in the benzene-induced genotoxicity, myelotoxicity, and leukemia.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(628)

**Type:** Metabolism

**Remark:** Hepatocytes isolated from the adult male NMRI mouse or Wistar rat were incubated for 1 h with 0.5 mM <sup>14</sup>C-benzene, the supernatant was separated from the cells, and analysed for benzene metabolites. Separately, formation of sulphate conjugates during benzene metabolism was studied in hepatocytes in the presence of <sup>35</sup>S-sulphate. In addition sulphate conjugation of the benzene metabolites hydroquinone and 1,2,4-trihydroxybenzene was investigated in mouse liver cytosol supplemented with 3'-phosphoadenosine-5'-phospho-<sup>35</sup>S-sulphate. Two novel metabolites, not detectable in rat hepatocyte incubations, were found in mouse hepatocytes, and were identified as 1,2,4-trihydroxybenzene sulphate and hydroquinone sulphate. Formation of the <sup>35</sup>S-labelled conjugates could be demonstrated in incubations of mouse liver cytosol with hydroquinone or 1,2,4-trihydroxybenzene supplemented with 3'-phosphoadenosine-5'-phospho-<sup>35</sup>S-sulphate, and in mouse hepatocytes incubated with benzene and <sup>35</sup>S-sulphate. In comparison with hepatocytes from the Wistar rat, hepatocytes from the NMRI mouse were almost three times more effective in metabolizing benzene. The higher formation of hydroquinone, and the formation of trihydroxybenzene

- sulphate and hydroquinone sulphate, mainly contributed to the higher rate of benzene metabolism.
- Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment
- 14-SEP-2000 (859)
- Type:** Metabolism
- Remark:** The effect of induction by phenobarbital (PB), beta-naphthoflavone (BNF), and benzene on benzene metabolism was studied in hepatic microsomes from male Sprague-Dawley rats. Two distinct forms of mixed-function oxidase activity appeared to metabolize benzene. One form was active at all substrate concentrations in microsomes from control, benzene-treated, and BNF-treated animals, and at benzene concentrations of 0.8 mM and below in microsomes from PB-treated animals. It was saturated at benzene concentrations above 0.4 mM, had a pH optimum of approximately 6.6, and was stimulated by fluoride. Pretreatment with benzene, but not BNF, increased benzene metabolism in these preparations. Benzene metabolism in microsomes from PB-induced rats was less active than in controls at benzene concentrations below 0.8 mM, but increased rapidly at higher benzene concentrations. Further characteristics of the PB-induced enzyme activity were that saturation was not observed at benzene concentrations as high as 4 mM, the pH optimum for benzene metabolism in these preparations was 7.1, metabolism was not stimulated by fluoride, and metabolism was inhibited by metyrapone. Both phenol and an unidentified polar component were formed from benzene in all microsomal preparations.
- Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment
- 14-SEP-2000 (903)
- Type:** Metabolism
- Remark:** The metabolic interactions of benzene and toluene co-exposure were investigated in male Fischer rats. Endosed recirculated exposure system was used to obtain inhalation uptake curves for individual chemicals as well as for a mixture of the two compounds. Pharmacokinetic parameters for benzene and toluene individually were determined in previous experimental studies. These values were incorporated into a physiologically based pharmacokinetic model which simulated the inhalation uptake process for both chemicals simultaneously. An optimal fit to the uptake curves for simultaneous exposure was obtained by adjusting the metabolic interaction terms for each chemical. Mutual suppression of metabolism was apparent. Toluene more effectively inhibited benzene metabolism than the reverse.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (911)

**Type:** Metabolism

**Remark:** Alpha, beta-unsaturated aldehydes are known to react with SH-containing compounds. The kinetics for the reaction of trans,trans-muconaldehyde with glutathione was investigated. The rate of the reaction was followed by measuring the decrease in optical density at 272 nm, the major absorption band of muconaldehyde which disappears after 1,2-addition of glutathione. The stoichiometry of the reaction obtained by measuring the amount of muconaldehyde and glutathione consumed at t=30 min was 1:1 on a molar basis in tris buffer. It has been suggested that the rate of reaction between alpha, beta-unsaturated aldehydes with sulfhydryl compounds is directly proportional to their toxicity. In comparison with the literature on toxic alpha beta-unsaturated aldehydes, the data show that muconaldehyde is less reactive than presumed toxic lipid peroxide decomposition products of this nature.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(922)

**Type:** Metabolism

**Remark:** The role of cell-specific metabolism in benzene toxicity was examined in both murine and human bone marrow. The selective toxicity of hydroquinone at the level of the macrophage in murine bone marrow stroma may be explained by a high peroxidase/NAD(P)H:quinone oxidoreductase (NQO1) ratio. Peroxidase metabolize hydroquinone to the reactive 1,4-benzoquinone whereas NQO1 reduces the quinones formed, resulting in detoxification. Peroxidase and NQO1 activity in human stromal cultures vary as a function of time in culture with peroxidase activity decreasing and NQO1 activity increasing with time. Peroxidase activity, and more specifically MPO, which had previously been considered to be expressed at the promyelocyte level, was detected in murine lineage-negative and human CD34+ progenitor cells. This provides a metabolic mechanism whereby phenolic metabolites of benzene can be bioactivated in progenitor cells, which are considered to be initial target cells for the development of leukemias. Consequences of a high peroxidase/NQO1 ratio in HL-60 cells were shown to include hydroquinone-induced apoptosis. Hydroquinone can also inhibit proteases known to play a role in induction of apoptosis, suggesting that it may be able to inhibit apoptosis induced by other stimuli. Modulation of apoptosis may lead to aberrant hemopoiesis and neoplastic progression.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(962)

**Type:** Metabolism

**Remark:** To determine the effect of exposure concentration and the route of administration on benzene metabolism, male F344/N rats and B6C3F1 mice were orally exposed to 1, 10, and 200 mg benzene/kg, and by inhalation for 6 hr to 5, 50, and 600 ppm benzene vapor. The effect of different exposure rates on the metabolism of benzene was determined by exposing rodents over different time intervals to the same total amount of benzene [constant concentration x time factor (C x T) = 300 ppm.hr]. Water-soluble metabolites constituted > 90% of the metabolite dose to the tissues and were used as a measure of the metabolism of benzene via different pathways. Water-soluble metabolites were measured in blood, urine, liver, lung, and bone marrow from animals killed following oral exposures and during and following inhalation exposures. The total "dose" to the tissue of individual metabolites was determined by the area under the curve (AUC). The results indicated a shift in metabolism from putative toxification pathways to detoxification pathways as the exposure concentration or oral dose increased. In mice, hydroquinone glucuronide and muconic acid (markers of toxification metabolic pathways) represented a greater percentage of the administered dose at low doses than at high doses. At high doses, phenylglucuronide and prephenylmercapturic acid (detoxification products) increased as a percentage of the administered dose. This same metabolic shift was observed in rats, except that hydroquinone glucuronide was a minor metabolite of benzene at all concentrations. The AUC of phenylsulfate (detoxification pathway) was proportional to the exposure concentration in both species. Within the range of C x T factors studied, the rate of the inhalation exposure to benzene did not affect the AUC of metabolites in tissues of rats; however, a high dose rate (600 ppm 0.5 hr) in mice caused a shift in metabolism to phenyl conjugates. The comparison of oral and 6-hr inhalation exposures indicated that, in terms of metabolite dose to tissues, there is no simple relationship between these two routes of administration. An oral dose and an inhalation exposure concentration which produce an equal dose of one metabolite produce very different doses of another metabolite. These studies demonstrated a species difference in benzene metabolism, as well as a metabolic shift in benzene metabolic pathways as the exposure concentration was increased.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Flag:** Risk Assessment

06-JAN-1997

(985)

**Type:** Metabolism

**Remark:** The metabolism of [14C]benzene by cynomolgus monkeys and chimpanzees, animals phylogenetically closer to humans than rodents, was studied. Monkeys were dosed ip with 5, 50, or 500 mg [14C]benzene/kg body wt. Urine was collected for up to 24 hr following exposure and was analyzed for benzene metabolites. The proportion of the administered 14C excreted in the urine of monkeys decreased from approximately 50 to 15% as the dose increased. Phenyl sulfate was the major urinary metabolite. The proportion of hydroquinone conjugates and muconic acid in the monkey's urine decreased as the dose increased. The proportion of catechol conjugates was not affected by dose. The proportion of muconic acid was consistently much lower in the monkey than in the mouse or rat. Three chimpanzees were administered 1 mg [14C]benzene/kg body wt. iv; essentially all of the injected 14C was recovered in the urine. Of the total urinary metabolites, 79% were accounted for by phenyl conjugates and less than 15% by hydroquinone conjugates or muconic acid. Catechol conjugates were not detected. The metabolism of benzene appeared to be qualitatively similar but quantitatively different in the species studied. The mouse, the sensitive rodent species, forms the highest levels of hydroquinone conjugates and muconic acid and the chimpanzee, the lowest. In all animal species studied for the effect of dose on benzene metabolism, as the dose decreased, a larger proportion of the benzene metabolites was represented by hydroquinone conjugates and muconic acid.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(986)

**Type:** Metabolism

**Remark:** Removal of 70-80% of the liver reduced both the metabolism and the toxicity of benzene in rats. Metabolism was evaluated by measuring the levels of urinary metabolites in both sham-operated and partially hepatectomized rats given 2200 mg/kg [3H]benzene sc. Toxicity was evaluated by measuring the incorporation of 59Fe into circulating erythrocytes. The observation that partial hepatectomy decreases benzene metabolism and protects against benzene toxicity indicates that the liver may play a primary role in the development of benzene-induced bone marrow toxicity. The fact that benzene administration also reduces the ability of the liver to regenerate after partial hepatectomy suggests that the regenerating liver may serve as a model system in lieu of the bone marrow for studying the mechanism by which benzene inhibits cell proliferation.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(995)

**Type:** Metabolism

**Remark:** When benzene or toluene was administered to rats ip in combination with the other, their disappearance rate from blood and the rate of urinary excretion of their metabolites were delayed compared with those when they were given separately. This metabolic interaction was found to be dose dependent. The metabolism of benzene or toluene studied in vitro with rat liver 10,000g supernatant fraction was inhibited competitively by the presence of the other. The solubility of benzene and toluene in blood and their binding with bovine serum albumin were not influenced by the presence of the other, indicating that absorption and distribution are unaffected by their simultaneous presence. A human experimental exposure to a mixture of benzene and toluene revealed that there is no significant interaction between them with respect to their fate.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1004)

**Type:** Metabolism

**Remark:** Chronic exposure to benzene (25 to 1000 ppm) is characterized by a progressive degeneration of bone marrow and dysfunction of the hemopoietic system. In the peripheral blood, lymphocytopenia is one of the most sensitive and easily measured indicators of benzene toxicity. Extensive studies have provided considerable evidence that the expression of benzene toxicity requires metabolism of the parent compound to one or more toxic species including phenol, catechol, and hydroquinone. In the bone marrow, a putative reaction pathway for the formation of covalent adducts from benzene requires myeloperoxidase.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1007)

**Type:** Metabolism

**Remark:** The metabolic pathways and kinetics of benzene metabolism were explored in vitro, providing sufficient data to develop a mathematical model of the reactions that occur. Differences in in vitro benzene metabolism among mice, rats, and individual humans were investigated. Mice metabolize benzene faster than rats, while the range of rates exhibited by human tissue samples spans that of mice and rats. Some qualitative differences were observed between in vitro and in vivo benzene metabolism. These differences can be explained, however, by incorporating the regional distribution of liver enzymes into a physiologically based pharmacokinetic (PBPK) model.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1016)

**Type:** Metabolism

**Remark:** The metabolism of two of benzene's phenolic metabolites, phenol and hydroquinone, by peroxidase enzymes was studied in detail. Studies employing horseradish peroxidase and human myeloperoxidase have shown that in the presence of hydrogen peroxide phenol is converted to 4,4'-diphenoquinone and other covalent binding metabolites, whereas hydroquinone is converted solely to 1,4-benzoquinone. Phenol stimulates the latter conversion rather than inhibiting it, an effect that may play a role in the in vivo myelotoxicity of benzene. Repeated coadministration of phenol and hydroquinone to B6C3F1 mice resulted in a dramatic and significant decrease in bone marrow cellularity similar to that observed following benzene exposure. A mechanism of benzene-induced myelotoxicity is proposed in which the accumulation and interaction of phenol and hydroquinone in the bone marrow and the peroxidase-dependent formation of 1,4-benzoquinone are important components. This mechanism may also be responsible, at least in part, for benzene's genotoxic effect, as 1,4-benzoquinone has been shown to damage DNA and is shown here to induce multiple micronuclei in human lymphocytes. Secondary activation of benzene's phenol metabolites in the bone marrow may therefore play an important role in benzene's myelotoxic and carcinogenic effects.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1059)

**Type:** Metabolism

**Remark:** Macrophages are more sensitive than fibroblastoid stromal cells (LTF cells) to the toxic effects of the benzene metabolite hydroquinone. The role of selective bioactivation and/or deactivation in the macrophage-selective effects of hydroquinone was examined. LTF and macrophage cultures were incubated with 10  $\mu$ M [ $^{14}$ C]hydroquinone to examine differential bioactivation. After 24 hr, the amount of  $^{14}$ C covalently bound to acid-insoluble macromolecules was determined. Macrophages had 16-fold higher levels of macromolecule-associated  $^{14}$ C than did LTF cells. Additional experiments revealed the hydroquinone bioactivation to covalent-binding species was hydrogen peroxide dependent in macrophage homogenates. Covalent binding in companion LTF homogenates was minimal, even in the presence of excess hydrogen peroxide. These data suggest that a peroxidative event was responsible for bioactivation in macrophages and, in agreement with this, macrophages contained detectable peroxidase activity whereas LTF cells did not. Bioactivation of [ $^{14}$ C]hydroquinone to protein-binding species by peroxidase was confirmed utilizing purified human myeloperoxidase in the presence of hydrogen peroxide and ovalbumin as a protein source. High performance liquid chromatographic analysis of

incubations containing purified myeloperoxidase, hydroquinone, and hydrogen peroxide showed that greater than 90% of hydroquinone was removed and could be detected stoichiometrically as 1,4-benzoquinone. 1,4-Benzoquinone was confirmed as a reactive metabolite formed from hydroquinone in macrophage incubations using excess GSH and trapping the reactive quinone as its GSH conjugate, which was measured by high performance liquid chromatography with electrochemical detection.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1127)

**Type:** Metabolism

**Remark:** DBA/2 mice have been reported to be more susceptible than C57BL/6 mice to the bone marrow toxic effects of benzene. The activity of quinone reductase (QR) (NADPH:DT diaphorase), a quinone detoxifying enzyme, in whole bone marrow and bone marrow-derived stromal cells from these two strains of mice was studied. The sensitivity of bone marrow-derived stromal cells to toxicity induced by several metabolites of benzene was also investigated. Whole bone marrow and primary cultures of stromal cells cultured from DBA/2 mice had a lower basal level of QR activity compared to those of C57B1/6 mice and as such exhibited a greater sensitivity to the toxic effects of hydroquinone (HQ), a metabolite of benzene. However, there was no difference between the two strains of mice to benzoquinone- or phenol-induced toxicity. Increased QR activity in DBA/2 and C57B1/6 stromal cells could be induced by prior stromal cell treatment with tert-butylhydroquinone which resulted in protection against subsequent hydroquinone treatment. Differences in target organ QR activity may contribute to differential susceptibility to quinone-generating bone marrow toxins.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1173)

**Type:** Metabolism

**Remark:** Enzymes catalyzing the two-electron reduction of quinones to hydroquinones are thought to protect the cell against quinone-induced oxidative stress. Carbonyl reductase, a cytosolic, monomeric oxidoreductase of broad specificity for carbonyl compounds, was found to be the main NADPH-dependent quinone reductase in human liver, whereas DT-diaphorase, the principle quinone reductase in rat liver, contributed a very minor part to the quinone reductase activity of human liver. The purified enzyme from liver catalyzed the reduction of a great variety of quinones. The best substrates were benzo- and naphthoquinones with short substituents, and the K-region orthoquinones of phenanthrene, benz(a)anthracene, pyrene and benzo(a)pyrene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1245)

**Type:** Metabolism

**Remark:** Bone marrow stromal cells from mice were significantly more susceptible to the cytotoxicity induced by the benzene metabolites hydroquinone (HQ) and benzoquinone (BQ) than cells from rats. Since cellular glutathione (GSH) and quinone reductase (QR) are known to play critical roles in modulating HQ-induced cytotoxicity, the GSH content and the QR and glutathione S-transferase (GST) activity in stromal cells from both species was measured. In rat cells, the GSH content and the QR specific activity were 2 and 28 times as much as those from mice, respectively. GSH and QR in both mouse and rat stromal cells were inducible by 1,2-dithiole-3-thione (D3T). D3T pretreatment of both mouse and rat stromal cells resulted in a marked protection against HQ-induced toxicity. Pretreatment of both mouse and rat stromal cells with GSH ethyl ester also provided a dramatic protection against HQ-induced toxicity. Conversely, dicoumarol, an inhibitor of QR, enhanced the HQ-induced toxicity in stromal cells from both mice and rats, indicating an important role for QR in modulating HQ-induced stromal toxicity in both species.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1311)

**Type:** Neurotoxicity

**Remark:** methods: narcotic properties of benzene studied on 10 rabbits exposed to 3.5-4.5% benzene vapor in air.  
results:  
symptoms                      average time of occurrence in minutes  
light anesthesia, relaxed                      3.7  
excitation (running movement, tremor, chewing)                      5.0  
pupillary reflex lost                      6.5  
loss of blink reflex to tactual stimulus                      11.4  
pupillary contraction                      12.0  
involuntary blinking                      15.6  
death                      36.2

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** no data

06-JAN-1997

(177)

**Type:** Neurotoxicity

**Remark:** method: 5 male CD-1 mice per group were fed drinking water containing 0, 31, 166, or 790 mg/l benzene (analytical) for 28 d; at termination monoamine neurotransmitter concentrations in different brain regions were determined.  
results: no significant effects on body weight gain, water and food consumption; benzene doses correspond to 0, 8, 40, 180 mg/kg/d, respectively; dose dependent significant increase in norepinephrine (hypothalamus, medulla oblongata,

cerebellum), dopamine (corpus striatum, hypothalamus) and serotonin (hypothalamus, midbrain, corpus striatum, cerebral cortex, medulla oblongata) levels; also increased levels of catecholamine metabolites indicating induction of synthesis and catabolism of monoamine transmitters by benzene ingestion.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** purity 99.9%

06-JAN-1997

(535)

**Type:** Toxicokinetics

**Remark:** In summary, benzene is well absorbed in humans and experimental animals after oral and inhalation exposures, but in humans dermal absorption is poor. Approximately 50% absorption occurs in humans during continuous exposures to 163-326 mg/m<sup>3</sup> for several hours. After a 4-hr exposure to 170-202 mg/m<sup>3</sup>, retention in the human body was approximately 30%, with 16% of the retained dose having been excreted as unchanged benzene in expired air. Women may retain a greater percentage of inhaled benzene than men. Benzene tends to accumulate in tissues containing high amounts of lipids, and it crosses the placenta. Benzene is eliminated by exhalation of unmetabolized benzene from the lungs and by metabolism in the liver (lesser extent by bone marrow) and following excretion via urine.

Reliability: 4 (validity cannot be judged)

data from secondary literature, but validated (WHO, 1993) by an expert group (WHO task group)

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(874) (1252)

**Type:** Toxicokinetics

**Remark:** method: 6 male and 6 female human volunteers inhaled 52-62 ppm benzene for 4 h; benzene concentration was measured in 1-h-intervals by GC and spectrophotometry.  
results: retention decreased with duration of exposure and reached a constant level (30%, estimated) after 2 h (no differences between men and women); absorption was greatest during 1st 5 minutes of exposure; after 4 h 46.9% of the dose was taken up by the subjects, 16.8% was excreted as unchanged benzene in the expired air; metabolites of benzene were excreted in the urine mainly in form of sulfate and glucuronide conjugates of phenol.

Reliability: 2 (valid with restriction)

Comparable to guideline study with acceptable restrictions  
no data about test substance

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(838) (839)

**Type:** Toxicokinetics

**Remark:** method: rabbits received 340, 400 or 500 mg/kg  
14C-radiolabelled benzene by oral gavage and excretion in  
the urine and expired air was determined for up to 7 d after  
dosing.  
results: 90% of total radioactivity of applied dose was  
absorbed and eliminated in air and urine; oral absorption  
occurs readily and rapidly; 46.5% of the label was recovered  
as unchanged benzene in expired air (70 h, 400 mg/kg); 34.8%  
of the applied dose (48 h, 340 mg/kg) were excreted via  
urine, mainly in form of conjugated phenol (23.5%); other  
metabolites excreted were 4.8% hydroquinone, 2.2% catechol,  
0.3% 1,2,4-trihydroxybenzene, 1.3% muconic acid and 0.5%  
L-phenylmercapturic acid; 5 or 10% (500 or 340 mg/kg,  
respectively) of the labelled benzene remained in the  
tissues or were excreted in the faeces (0.88% or 0.3%,  
respectively) 48 h after dosing.  
Reliability: 2 (valid with restriction)  
Comparable to guideline study with acceptable restrictions  
no data about number of animals

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(869)

**Type:** Toxicokinetics

**Remark:** method: male F344/N rats or male B6C3F1 mice (n=3-4/dose)  
were a) gavaged with 0.5, 5, 15, 50, 150 or 300 mg/kg  
benzene (high dose only rats) and excretion determined next  
48 h or b) were exposed to 33, 75, 340, 680, 2260 mg/m<sup>3</sup>  
(rats) or 29, 75, 350, 2570 mg/m<sup>3</sup> (mice) for 6 h and  
excretion measured for up to 56 h after termination of  
exposure.  
results: a) absorption > 97% in mice and rats for doses  
0.5-150 mg/kg; at < 15 mg/kg > 90% excreted in urine, at >  
15 mg/kg increasing percentage exhaled unchanged (saturation  
of metabolic pathways); at > 50 mg/kg total metabolites not  
linearly related to administered dose; metabolites per unit  
body weight equal in mice and rats at doses up to 50 mg/kg,  
total metabolites not increased at higher dose in mice.  
b) absorption 33% and 15% in rats (low dose and high dose,  
respectively) and 50% and 10% in mice; total metabolite  
formation exponentially related to benzene concentration  
with one-half max. amount of metabolic formation occurring  
at 220 mg/m<sup>3</sup> for mice and 650 mg/m<sup>3</sup> for rats; mainly due to  
higher amount inhaled the total metabolites were higher in  
mice than in rats at any concentration.  
Reliability: 1  
Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Test substance:** 14C-labelled benzene, purity > 99%  
**Flag:** Risk Assessment

14-SEP-2000

(987)

**Type:** Toxicokinetics

**Remark:** method: 5 male Sprague-Dawley rats received dermal applications of <sup>14</sup>C-labelled benzene under a glass cap (evaporation prevented) for 72 h; benzene blood levels measured at 2, 4, 8, 12, 24, 30, 48, and 72 h; excretion via urine, faeces and expired air determined.  
results: max. plasma concentration at 8-24 h (ca. 2000 DPM/ml); absorption half-live 3.1 h and elimination half-live 23 h in rat plasma; excretion of radioactivity 86% in the urine, 0.2% in the faeces and 13% via expired air at 0-48 h; in 0-12 h urine 37.7% of total radioactivity excreted as phenol, 13.1% as catechol, 19.0% as hydroquinone and 5.4% as 1,2,4-trihydroxybenzene; after 48 h highest radioactivity was found in the kidney (0.026%), followed by 0.013% in the liver and 0.011% in the treated skin.  
Reliability: 1  
Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1044)

**Type:** Toxicokinetics

**Remark:** method: 3 female Sprague-Dawley rats per group received a single application of 0.15, 1.5, 15, 150, or 500 mg/kg <sup>14</sup>C-benzene by gavage and were sacrificed 1, 3, 6, 9, 12, and 24 h after administration for radioactivity analysis and determination of metabolites.  
results: highest levels of radioactivity in all tissues at 1 h (peak probably earlier); at the 2 lowest doses highest levels in liver and kidney (brain and fat not determined), intermediate level in blood and low levels in zymbal gland, nasal and oral cavity, and mammary gland; at 15 mg/kg disproportionate increases in mammary gland and bone marrow; biphasic elimination of the radioactivity, half-live in the rapid phase (determined at 0.15 mg/kg) between 2.2 h (blood) and 4.2 h (liver) and in the slow phase between 11 h (bone marrow) and 29 h (blood); slow phase half-live reduced with higher doses (analysed in blood and zymbal gland); no accumulation of radioactivity in zymbal gland with increasing doses; metabolites determined 1 h after dosing at 15 mg/kg (relative percentage): main metabolites in blood 83% phenyl sulfate and 64% hydroquinone, in liver 89% hydroquinone and 26% phenylsulfate, in kidney 65% hydroquinone, 26% phenol, 23% phenyl sulfate and 15% muconic acid.  
Reliability: 1  
Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Test substance:** <sup>14</sup>C-labelled benzene, purity >99%  
**Flag:** Risk Assessment

14-SEP-2000

(703)

**Type:** Toxicokinetics

**Remark:** method: 3 male F344 rats per group were exposed to a) 484 ppm (analytical concentration) for 0.5, 1, 1.5, 2, 4, 6, 8 h and sacrificed for steady state determination or they were b) exposed to 490 ppm (analytical conc.) for 6 h and sacrificed after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9 h post exposure period for elimination study.  
results: benzene concentration reached steady state within 4 h in blood, 6 h in fat, and < 2 h in bone marrow; highest steady state concentration in fat (164 ug/g) followed by bone marrow (37 ug/g) and kidney (25 ug/g); similar elimination half-lives in all tissues (0.4-0.8 h) except fat (1.6 h); benzene eliminated in the expired air in a biphasic manner, half-lives 0.7 h and 13.1 h; phenol disappeared rapidly from blood and bone marrow, whereas catechol and hydroquinone remained fairly constant over the time period studied.

Reliability: 1

Comparable to guideline study

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Test substance:** analytical grade

**Flag:** Risk Assessment

14-SEP-2000

(936)

**Type:** Toxicokinetics

**Remark:** The results of measurement of benzene in exhaled breath and conjugated phenol in urine show that for a human subject:  
1. The long-term retention and elimination of benzene is influenced by both the concentration and the duration of exposure. These variables appeared to be directly related to the multiple of the concentration and duration of exposure (here termed "exposure dose").  
2. The presence of other hydrocarbons at a concentration of about 150 ppm during an 8-hour exposure period did not appear to influence the uptake or metabolism of benzene at about 10 ppm.  
3. The amount of benzene absorbed and eliminated over a long period appears to depend directly on the energy expenditure of the subject. The elimination may also be influenced by energy expenditure after work. This was confirmed by sampling the breath during exercise in the early and middle phases of benzene elimination.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(1035)

**Type:** Toxicokinetics

**Remark:** Groups of AKR mice and Sprague-Dawley rats were exposed to either 300 or 100 ppm benzene for 6 hr/day x 5 days/week for 20 days. The uptake and clearance of benzene was followed in blood during and after the 1st, 6th and 20th exposures. In rats, the maximum concentrations of benzene

in blood (Cmax) were proportional to the exposure concentrations, that is the Cmax values at 300 ppm were three times the Cmax values at 100 ppm. This was not the case with the mice which showed Cmax values at 300 ppm equal to five to eight times the Cmax values at 100 ppm. At both exposure levels mice exhibited larger elimination rate constants than rats. Both rats and mice showed larger elimination rate constants at 100 ppm than at 300 ppm. At 300 ppm, the mice showed progressive increases in elimination rate constants, progressive decreases in Cmax and an unusual shift from monoexponential clearance to biexponential clearance between the 6th and 20th exposures. It is postulated that the induction of benzene-metabolizing enzymes and/or an increase in lipid tissue mass are responsible for the progressive changes of the kinetic parameters of the mice.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1069)

**Type:** other

**Remark:** Marrow stromal macrophage dysfunction and deficient interleukin-1 (IL-1) production has been reported for patients with severe aplastic anemia. The stromal macrophage, a target of benzene (BZ) toxicity, is involved in hematopoietic regulation through the synthesis of several cytokines including IL-1, which is required for development of myeloid stem cells and which induces cytokine production by stromal fibroblasts and from the pluripotent stem cell. We have demonstrated that hydroquinone (HQ), a major toxic metabolite of BZ in marrow, prevents the proteolytic conversion of 31 kDa pre-IL-1 $\alpha$  to the 17 kDa cytokine by calpain in lysates of purified murine stromal macrophages. Stromal macrophages from BZ-treated mice produce the 31 kDa pre-IL-1 $\alpha$  when stimulated in culture with endotoxin (LPS), but cannot convert the precursor to IL-1 $\alpha$ . This observation suggests that HQ also prevents the conversion *in vivo* resulting in BZ-induced bone marrow cell depression that can be bypassed by the concomitant administration of recombinant IL-1 $\alpha$  with BZ. In this report, we show that 1,4-benzoquinone (BQ), the oxidation product of HQ in the cell, causes a concentration-dependent inhibition of highly purified human platelet calpain. HQ also inhibits the processing of pre-IL-1 $\beta$  by IL-1 $\beta$  convertase (ICE). The addition of 2  $\mu$ M HQ to B1 cells that undergo autocrine stimulation by IL-1 $\beta$  resulted in the cessation of autocrine cell growth and IL-1 $\beta$  secretion into the culture medium, as determined by Western immunoblots of the culture supernatants. Purified ICE treated with 3  $\mu$ M BQ was incapable of converting 31 kDa recombinant pre-IL-1 $\beta$  to the 17 kDa mature cytokine as analyzed by polyacrylamide gel electrophoresis and Western immunoblotting. The increased apoptosis in hematopoietic progenitor cells of the hyperplastic marrow of BZ-induced aplastic anemia patients may result from a lack of essential

cytokines because of deficient IL-1 production caused by an inhibition by BQ of the sulfhydryl-dependent proteases required for the processing of pre-IL-1alpha and/or pre-IL-1beta to the active cytokines.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997 (823)

**Type:** other

**Remark:** Benzene exposure to smokers

**Source:** German rapporteur

**Flag:** Risk Assessment

13-SEP-2000 (517)

**Type:** other: Absorption

**Remark:** Benzene is readily absorbed via lung and about 40-50% is retained. It is taken up preferentially by fatty and nervous tissues and about 30-50% is excreted unchanged via lung; a 3-phase excretion pattern is seen at approx. 0.7-1.7h, 3-4h and 20-30h.

**Source:** REPSOL PETROLEO, S.A. MADRID

08-JUL-2005 (550)

**Type:** other: Absorption

**Remark:** Study measured the penetration of benzene through human abdominal skin in vitro from solutions in water, gasoline, hexadecane, and isooctane and found permeability constants which averaged 111.0, 1.4, 0.9, and  $3.7 \times 10^{-3}$  cm.h(-1), respectively. The stratum corneum/water partition coefficient for benzene has been measured and averages 30.0. The partition coefficients for the other vehicles are very low and cannot be measured by the method used for water. A new method is presented for calculating these coefficients, however, and they are 0.11, 0.14, 0.17, and 0.19 for gasoline, hexane, isooctane, and hexadecane. The flux of benzene through epidermis in vitro from air saturated with benzene at 31 degree C averages 1.0 ul cm(-2).h(-1).

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000 (110)

**Type:** other: Absorption

**Remark:** The percutaneous absorption of benzene was measured in the monkey, the miniature pig, and man using both in vitro and in vivo techniques. Following the application of a thin layer (5-10 ul/cm<sup>2</sup>) of benzene in vivo, absorption was found to average less than 0.2% of the applied dose in 11 species studied. Total absorption was 0.14% in the monkey, 0.09% in the mini-pig, and 0.05% in man. In vitro studies demonstrated rapid penetration of benzene through the skin.

When the same dose applied in vivo was used in vitro, the peak rate of absorption occurred at 15-40 minutes and total absorption was similar to that measured in vivo. Absorption was 0.19% in the monkey, 0.23% in the mini-pig, and 0.10% in man. Benzene absorption was found to be a function of its contact time with the skin. Application of progressively larger doses which persisted on the skin for up to 3 hours resulted in 10-100 times greater absorption. Total absorption was found to be directly related to the length of time benzene remained on the skin.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(369)

**Type:** other: Absorption

**Remark:** Dermal absorption of liquid benzene was evaluated in a human volunteer. Benzene was applied at a dose of 0.06 g/cm<sup>2</sup> to 35-43 cm<sup>2</sup> of the forearm for 1.25-2 hours. The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin under conditions of complete saturation was calculated to be relatively low at approximately 0.4 mg/cm<sup>2</sup>/hour.

**Source:** Deutsche Shell Chemie GmbH Eschborn

18-AUG-2000

(472)

**Type:** other: Absorption

**Remark:** The permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane was studied using excised skin in a flow-through diffusion cell. The rate of resorption was determined by measuring the amount of substance found in the receptor fluid beneath the skin at steady-state. The rates of resorption (ug.cm<sup>-2</sup>.hr<sup>-1</sup>) were: benzene 99, ethylene glycol 118, formaldehyde from a concentrated solution of formalin 319, formaldehyde from a solution of 10% formalin in phosphate buffer 16.7, and n-hexane 0.83. The amount of substance in the skin at steady-state and after 0.5 hr of exposure was also determined. For all substances, the sum of the amount in the receptor medium and in the skin at steady-state, were larger than the amount obtained by multiplying the resorption rate by the time of exposure. For benzene, ethylene glycol and n-hexane the amount absorbed during the first half-hour of exposure was considerably larger than the amount resorbed during a same unit of time at steady-state. These data call attention to the fact that the absorption rate is higher before steady state is attained.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(699)

**Type:** other: Absorption

**Remark:** Penetration of benzene through the skin of the rhesus monkey was determined using  $^{14}\text{C}$ -benzene, and quantitating the labelled metabolites in urine. The modes of application and amounts of benzene that penetrated the skin (indicated in parentheses) are as follows: (1) a single, direct cutaneous application of liquid benzene (0.172 +/- 0.139%); (2) a single application of benzene-containing [0.36%] solvent (0.0805 +/- 0.0306%); (3) multiple washes with full strength benzene (0.848 +/- 0.0806%); (4) multiple washes with the benzene-containing [0.35%] solvent (0.431 +/- 0.258%); (5) removal of the stratum corneum followed by application of full-strength benzene (0.909 +/- 0.627%); and (6) application of benzene to the palmar surface (0.651 +/- 0.482%). Benzene penetration in the monkey may be used to estimate penetration in man, both for industrial hygiene purposes and general toxicological use.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(727)

**Type:** other: Absorption

**Remark:** This study investigated the whole-body dermal penetration of styrene, xylene, toluene, perchloroethylene, benzene, halothane, hexane, and isoflurane in rats and compared the permeability constants with available human studies on vapor penetration. Rats with closely clipped fur were exposed to organic chemical vapors (3000 to 60,000 ppm) while breathing fresh air through a latex mask. Blood concentrations taken during the 4-hr exposures were determined by sampling through indwelling jugular cannulas. A physiologically based pharmacokinetic model was used to predict permeability constants consistent with the experimental blood concentrations. Permeability constants (cm/hr) were estimated by a least-square optimization and ranged from 1.75 cm/hr for styrene to 0.03 cm/hr for isoflurane. The value for benzene was calculated to be 0.08 cm/hr. Rat permeability constants were uniformly two to four times greater when compared to the human constants which were calculated from the literature. These results indicate that organic vapor permeability constants in rats are a conservative estimate of organic vapor permeability constants in humans and that the consistent differences in permeability constants between these two species may be due to physiological differences in the skin.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(753)

**Type:** other: Absorption

**Remark:** Benzene concentrations in inhaled and exhaled air and in blood and urine were followed for 2 to 3 hr. in 27 experimental studies on 23 human subjects who inhaled benzene vapors in concentrations of 150 to 350 ug. per liter(47 to 100 ppm). The rate of absorption of benzene is highest in the first few minutes of inhalation (70 to 80 percent); afterward it drops quickly and in the majority of cases stays at 50 percent during the test period. During the test period an equilibrium between blood and air levels of benzene was not achieved. In the desaturation period 30 to 50 percent of the absorbed benzene is eliminated by the lungs, while the quantity of benzene eliminated by the kidneys is insignificant (0.1 to 0.2 percent), and the elimination continues for a long time. Benzene which is not excreted by the lungs or the kidneys remains in the body and is metabolized (50 to 70 percent, exceptionally more). On the basis of the experimental results, the decrease of the quantities of benzene in the blood and the exhaled air was calculated during the desaturation period, and it was found that both quantities are related to the actual quantity of benzene in the blood, according to a logarithmic curve. The ratio between the rate constants of desaturation in blood and exhaled air is near to 1.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(1082)

**Type:** other: Absorption

**Remark:** Nude mice each attached to a respirator to avoid pulmonary uptake were exposed in a glass exposure chamber to 200, 1000 or 3000 ppm of benzene, for 2, 4 or 6 h. The animals were killed at the end of the study and the amount of benzene retained in the whole body was determined by gas chromatography. Skin absorption rates were calculated from the amount retained in the whole body using the single compartment model (elimination rate constant) obtained in a previous experiment. There was a linear relationship between the amount of skin absorption and exposure time, and also a linear relationship between the skin absorption rate and concentration of exposed vapors. Skin absorption of benzene occurs by passive diffusion as defined by Fick's law. The skin absorption coefficient (cm/h) of benzene vapor was calculated by dividing the skin absorption rate by exposure concentration; the value was 0.619 for benzene. The coefficient may be useful for evaluating the amount of skin absorption of solvent vapors in the work environment.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(1164)

**Type:** other: Biomarkers

**Remark:** Results of experiments have shown that benzene is metabolized by animals in part to an intermediate that binds to cysteine groups in hemoglobin to form the adduct S-phenylcysteine (SPC). SPC in hemoglobin may be an effective biological marker for exposure to benzene. SPC was not detected in the globin of humans occupationally exposed to benzene concentrations as high as 28 p.p.m. for 8 h/day, 5 days/week. Also, examined the binding of benzene to cysteine groups of a different blood protein, albumin. SPC in the albumin of F344/N rats exposed by gavage to 0-10,000 umol/kg benzene was determined. Amounts of albumin-associated SPC increased as a function of dose, followed by a leveling off in the amount of SPC seen at doses > 1000 umol/kg. Levels of SPC were measured in humans occupationally exposed to average concentrations of 0, 4.4, 8.4 and 23 p.p.m. benzene 8 h/day, 5 days/week. Of nine controls, seven had levels of SPC below the limit of detection (0.1 pmol SPC/mg albumin). SPC increased in the exposed groups linearly, giving a statistically significant slope ( $P < 0.001$ ) of  $0.044 \pm 0.008$  pmol/mg albumin/p.p.m.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(87)

**Type:** other: Biomarkers

**Remark:** Structural chromosome aberrations and sister chromatid exchanges (SCEs) in peripheral blood were studied in female workers employed in the shoe-making industry in two periods: 1987 (group I; N = 38) and 1992 (group II; N = 45). Occupational exposure to benzene and toluene was confirmed through their determination in the working area, blood, and phenol in pre- and post-shift urine. The results were compared with those from the control group (N = 35). Benzene in the working atmosphere was significantly higher in 1987 compared to 1992, but was always lower than the current Croatian permissible concentration of 50 mg m<sup>-3</sup>. A statistically significant difference was also found in biological markers of benzene exposure between the two periods of the investigation. Increased absorption in the first period occurred because of intensified production in 1987, and this decreased significantly in 1992.

The cytogenetic study showed a significant increase in dicentric chromosomes in exposed groups I and II when compared to the control group. Statistically significant higher SCE frequencies were found in group I compared to the control group and also compared to group II. SCE frequencies were significantly higher in 1987 when greater benzene absorption occurred, confirmed by biomarkers of benzene exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(600)

**Type:** other: Cardiotoxicity

**Remark:** Two ml of benzene by mouth may produce symptoms, and 10 ml may be fatal. Inhalation of 100 ppm for several hours causes headache and a sense of fatigue; 1500 ppm for an hour causes marked depression, 3000 ppm is irritating to the eyes and nose on but a few minutes' exposure and 7500 ppm for half an hour or 20,000 ppm for a few minutes may cause death. The symptoms are due to gastric irritation and to depression of the central nervous system, myocardium and bone marrow. The heart beat becomes weak and irregular. Many acute deaths are due to ventricular fibrillation due to effort and release of epinephrine.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1123)

**Type:** other: Exposure

**Remark:** The authors found experimentally that benzene in food and beverages is present at levels equal or less than 2 ng/g.

**Source:** German rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(758)

**Type:** other: Exposure

**Remark:** For service station attendants, an 8-hour exposure has been measured to account to a maximum level of 3.34 ppm (=121  $\mu\text{g}/\text{m}^3$ ) in 1986. During later years, the concentrations were lower.

**Source:** German rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(233)

**Type:** other: Exposure

**Remark:** Benzene in seafish (Japan) were studied which were fished nearby refineries. Fish caught in Japanese sea water near refineries was tainted with benzene.

**Source:** German rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(855)

**Type:** other: Gynecological

**Remark:** Menstrual disorders characterized by profuse or scanty periods and dysmenorrhea occurred in 12 of 30 women who were occupationally exposed to benzene. Air measurements of benzene levels were not reported. Ten of the twelve women were married. Two of the ten women had spontaneous abortions and no births occurred in this group. This study did not assess the possibility of infertility. Gynecological exams revealed that scanty menstruation of 5

- of the patients were due to ovarian atrophy.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (1205)
- Type:** other: Hematotoxicity
- Remark:** This paper examined the effect of benzene and its metabolites, (phenol, hydroquinone and catechol) alone and in combination, on bone marrow function in female mice using  $^{59}\text{Fe}$  uptake as a measure of erythropoiesis. Benzene treatment at doses of 150 and 300 mg/kg resulted in dosage dependent reductions in radioiron uptake to 63% and 24%, respectively. Hydroquinone was marginally effective, but the inhibition occurred only at the highest dose tested (100 mg/kg). The combination of phenol and hydroquinone was more effective in reducing erythrocyte production than either chemical given alone. Catechol given alone was not inhibitory but when phenol was added to catechol, erythropoiesis was suppressed, as observed for the phenol and hydroquinone combination. These data suggest that benzene toxicity may be the result of cooperative inhibitory effects produced by its metabolites.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (460)
- Type:** other: Hematotoxicity
- Remark:** The present study evaluated whether other benzene metabolites including muconaldehyde (MUC), hydroquinone (HQ) and p-benzoquinone (BQ) could effect hematopoiesis either singly or in combination. Using radio-iron uptake into erythrocytes as a measure of hematopoiesis, it was demonstrated that BQ and MUC are potent inhibitors of bone marrow function in female mice. These two benzene metabolites reduced iron uptake at dosages of  $< 5-6 \text{ mg kg}^{-1}$ . The combination of MUC and HQ ( $100 \text{ mg kg}^{-1}$ ) was additive, reducing iron incorporation to an extent that was the sum of the effect of each chemical given alone. The combined effect of MUC and BQ was significantly less than additive, demonstrating antagonism in the response. Multiple regression was used to study the contributions of the components of binary mixtures of the benzene metabolites. The study results support the hypothesis that the toxic effects of benzene are produced by several metabolites acting interactively.
- Source:** Deutsche Shell Chemie GmbH Eschborn
- 06-JAN-1997 (461)

**Type:** other: Hematotoxicity

**Remark:** This study evaluated the effects of benzene (BZ) and hydroquinone (HQ), a BZ metabolite, on the human HL-60 promyelocytic leukemic cell line. Because the HL-60 cell is bipotential and can be induced to differentiate to monocytes or granulocytes, it has been used in many studies as a surrogate for the granulocyte/macrophage committed cell, GM-CFU. Treatment of HL-60 cells with BZ specifically induced differentiation along the granulocytic lineage as measured by morphology, induction of superoxide production and chloroacetate esterase activity and the appearance of the L12-2 surface antigen. Differentiation was induced via the activation of protein kinase C and phosphorylation of several proteins known to be involved in HL-60 cell differentiation. Subsequent to kinase C activation, arachidonic acid was released and the 5-lipoxygenase pathway was activated for the production of leukotriene D4 (LTD4) required for granulocytic differentiation. BZ induction of granulopoiesis was prevented by preincubation of HL-60 cells with inhibitors of protein kinase C. Treatment of HL-60 cells with tetraphorbol myristate acetate (TPA), 1 alpha, 25-dihydroxyvitamin D3 (1,25-(OH)2D3) or interleukin-1 (IL-1) induced HL-60 cells to differentiate to monocytes/macrophages. Hydroquinone prevented induction to monocytes/macrophages induced by TPA or 1 alpha, 25-dihydroxyvitamin D3, but not by IL-1 and significantly, had no effect on the induction of granulocytic differentiation by BZ or any other inducer. Taken together, these results indicate that BZ induces differentiation of HL-60 cells specifically to granulocytes by the activation of the lipoxygenase pathway and the production of LTD4. HQ prevents differentiation to monocytes/macrophages. Consequently, in the bone marrow, BZ could increase the number of granulocytic precursors and even granulocytes while at the same time HQ prevents the development of monocytes and macrophages. Although the findings have been obtained with a single cell line, they may provide some insight into the processes that occur during the differentiation of the GM-CFU, the normal bipotential myeloid committed progenitor.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(594)

**Type:** other: Hematotoxicity

**Remark:** The importance of cell proliferation in chemical carcinogenesis has been appreciated for over a decade. In tissues such as bone marrow with normally high rates of cell division, proliferation is tightly coordinated with cell differentiation. It has been established that survival, proliferation and differentiation of early hematopoietic progenitor cells (HPC) depends on the growth factors, interleukin 3 (IL-3) and/or granulocyte-macrophage colony stimulating factor (GM-CSF) and their synergism with other cytokines. In this study using both in vivo and in vitro

methods, the authors demonstrated increased recruitment of cells responsive to rGM-CSF following exposure to a variety of agents with documented leukemogenic potential. These findings support the possibility that transient alterations in HPC differentiation may be an important factor in the early stages of development of leukemia secondary to chemical or drug exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(566)

**Type:** other: Hematotoxicity

**Remark:** This research involved exposure of CBA/Ca male mice to benzene vapor in varying concentrations. Exposure to 300 ppm 6 hrs/day, 5 days/week, for 16 weeks is highly leukemogenic. Exposure for the same time to 100 ppm is also leukemogenic. Concentrations from 25 ppm to 400 ppm 6 hrs/day, 5 days/week, for 10 exposures produce an increasing lymphopenia. Exposure to 100 ppm for the same exposure time produces anemia, decrease in stem cell content of marrow, and marrow cellularity.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(258)

**Type:** other: Hematotoxicity

**Remark:** Effects of benzene inhalation on mouse pluripotent hemopoietic stem cells were evaluated. Male mice 8-12 wk old were exposed to 400 ppm benzene for 6 h/d, 5 d/wk, for up to 9 1/2 wk. At various time intervals exposed and control animals were killed, and cardiac blood was evaluated for changes in white blood cell (WBC) and red blood cell (RBC) content. In addition, femora and tibiae were evaluated for total marrow cellularity, stem cell content (as measured by the spleen colony technique), and the percent of stem cells in DNA synthesis (as determined by the tritiated thymidine cytocide technique). Exogenous spleen colonies grown from marrow of exposed animals were counted, identified, and scored by histological type. Exposure to benzene caused significant depressions of RBCs and WBCs throughout the exposure period, which continued for at least 14 d after exposure. Bone marrow cellularity and stem cell content were also depressed in exposed animals throughout the study. Tritiated thymidine cytocide of spleen colony-forming cells was generally increased in exposed animals, perhaps indicating a compensatory response to the reduction of circulating cells. Spleen colonies of all types were depressed after exposure to benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(259)

**Type:** other: Hematotoxicity

**Remark:** Groups of 40 rats were exposed to benzene vapor concentrations of approximately 4 or 5 days/week 830, 65, 60, 47, 44, 30, and 15 ppm for 5 hours per day, for periods ranging from 5 weeks to 7 months. A significant leucopenia resulted after 2-4 weeks of exposure to 830, 65 and 60 ppm, respectively. Exposure to 47 and 44 ppm induced a moderate but definite leucopenia after 5-8 weeks of exposure. The number of red blood cells and the concentration of hemaglobin in the circulating blood were not affected by these exposures. Exposure to 30 ppm of benzene (4 months), 30 ppm (3 months) and 15 ppm (7 months) did not induce changes in hemopoietic tissue.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(288)

**Type:** other: Hematotoxicity

**Remark:** Male B6C3F1 mice were exposed to 0, 1, 10, 100, or 200 ppm benzene by inhalation for 6 hr/day, 5 day/week for 1, 2, 4, or 8 weeks. At each sampling time, primitive and committed progenitor cells, differentiating and maturing lineage specific cells, and stromal cells in the bone marrow; T and B lymphocytes of the spleen and thymus; micronucleated reticulocytes and erythrocytes; and standard blood parameters were evaluated. At 100 and 200 ppm benzene, there were rapid and significant reductions in number of reticulocytes in the blood, B lymphocytes in the bone marrow and spleen, and an increased frequency of micronucleated reticulocytes in the bone marrow. At 10 ppm, the only parameter affected was a transient reduction in the number of splenic B lymphocytes. There were no significant effects induced by 1 ppm benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(347)

**Type:** other: Hematotoxicity

**Remark:** Several schedules of benzene exposure were evaluated for their effects on peripheral white blood cell counts, bone marrow cellularity and transplantable colony forming units (CFU-S) in male C57 B1/6 mice. Intermittent exposure to 4000 ppm benzene in air produced leukopenia without altering the bone marrow cellularity. This same treatment, however, decreased the number of CFU-S to 30% of control values. Uninterrupted exposure to lower levels of benzene decreased peripheral cell counts within 24 h, and later decreased marrow cellularity. Exposure of a non-dividing population of stem cells (CFU-S) to benzene for up to 24 h produced no detectable effect on the subsequent development of spleen colonies, suggesting that the effect of benzene on CFU-S occurs only after peripheral cells are depleted. These findings indicate that benzene has effects on both differentiated cells and undifferentiated stem cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(410)

**Type:** other: Hematotoxicity

**Remark:** Inhaled benzene hematotoxicity to recognize stem cell progeny was studied in male CD-1 mice exposed for 6 hr/day x5 days to one of the following approximate benzene concentrations: 1.0, 10, 100, 300, 600, 1280, 2400, and 4860ppm. Additional groups of mice were exposed for 6 hr/day x 5 days/week x 10 weeks to 9.6 ppm, or 6hr/day x 5 days/week x 26 weeks to 300 ppm. Following the 5-day exposures, granulocytopenia and lymphocytopenia were observed at levels  $\geq 100$  ppm with no change in WBC differential. RBC counts were depressed only at the two highest exposure levels while hematocrits were variably affected and showed no clear dose/response effect. Marrow and splenic cellularities were reduced at all levels  $\geq 100$  ppm. Marrow lymphocytes, splenic lymphocytes, and marrow granulocytes were reduced in accordance with the reduction in total cellularity, however, splenic granulocytes and spleen weights were depressed at almost all exposure levels. Nucleated RBCs in the marrow and spleen were depressed at all levels  $\geq 100$  ppm. Exposure for 50 days to 9.6 ppm benzene, a total dose equivalent to that delivered over 5 days at 100 ppm, induced no detectable changes in the peripheral blood or bone marrow.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(439)

**Type:** other: Hematotoxicity

**Remark:** In order to investigate the interaction of inhaled benzene with hematopoietic stem cells, marrow and spleen cells from male CD-1 mice were assayed for CFU-S by the spleen colony method, and for GM-CFU-C by an in vitro agar technique following benzene exposure using a number of regimes. Specifically, these consisted of a 6 hr/day x 5 days exposure to either 1, 10, 100, 300, 600, 1280, 2400, or 4860ppm (Experiment 1); a 6 hr/day x 5 days/week x 50 days exposure to 9.6 ppm (Experiment 2); and a 6 hr/day x 5 days/week x 26 weeks exposure to 300 ppm (Experiment 3). In Experiment 1 femoral and splenic cellularities were significantly reduced at concentrations  $\geq 100$  ppm. Marrow concentrations of GM-CFU-C was equivalent to or greater than control values at all levels, however, splenic GM-CFU-C concentration was decreased at 100 ppm and above. Femoral and splenic CFU-S and GM-CFU-C per organ were depressed at 100 ppm and above. Experiment 2 was designed to compare the effects of a 10-ppm exposure delivered over 50 days with the 100-ppm exposure delivered over 5 days. In Experiment 2, no detectable changes were observed in bone marrow, but splenic cellularity and the number and concentration of splenic CFU-S were elevated vs matched control. Experiment 3 repeated a regime that produced two

cases of myeloid leukemia in CD-1 mics and a marked depression was observed in marrow and spleen cellularity. The concentration and number of marrow and spleen CFU-S and marrow GM-CFU-C were also depressed. The number of splenic GM-CFU-C were also reduced, however, splenic GM-CFU-C concentration was increased relative to control. Stem cell depletion seems, therefore, to be involved in the pathogenesis of benzene-induced hematotoxicity.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(440)

**Type:** other: Hematotoxicity

**Remark:** Benzene (BZ) and Hydroquinone (HQ) were shown to induce granulopoiesis in a mouse model and in the murine diploid, interleukin (IL)-3-dependent, G-CSF-inducible, myeloblastic cell line, 32DcI3(G). We show that granulocyte-colony stimulating factor (G-CSF) can provide both a proliferative and differentiative signal for myeloblasts whereas BZ, HQ and LTD4, a G-CSF-induced effector essential for granulocytic differentiation, can replace G-CSF for differentiation, but cannot obviate the IL-3 requirement for growth and survival. HQ and LTD4 synergize with IL-3 to promote a significant stimulation of proliferation. An analysis of the kinetics of stage-specific granulocytic differentiation induced by these agents over 6 days in the absence of IL-3 demonstrated that all of the inducers caused virtually complete differentiation. G-CSF promoted predominantly terminal differentiation. BZ, like LTD4, showed a stimulation of all stages of progenitors as well as terminally differentiated granulocytes but with a delayed onset because BZ must induce the formation of LTD4. In contrast, HQ induced differentiation which arrested at the myelocyte stage.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(489)

**Type:** other: Hematotoxicity

**Remark:** Pregnant Swiss Webster mice were exposed from Day 6 through Day 15 of gestation to either air, 5 ppm, 10 ppm, or 20 ppm benzene. On Day 16 of gestation, 2 days after birth, and 6 weeks after birth, progeny of the exposed dams were assayed for the amount and type of hemoglobin produced and for recognizable hematopoietic cells in the peripheral blood and hematopoietic organs. None of the benzene exposures induced significant changes in the indices assayed from the 16-day fetuses. In contrast, 2-day neonates exposed in utero to all concentrations of benzene exhibited reduced numbers of circulating erythroid precursor cells. In addition, those 2-day neonates exposed in utero to 20 ppm benzene exhibited increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week adult mice exposed in utero to 20 ppm benzene

exhibited a similar pattern of enhanced granulopoiesis. These animals exhibited elevated numbers of splenic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of marrow erythropoietic precursor cells. These results suggest that in utero exposures to low concentrations of benzene can induce persistent enhanced production of recognizable granulopoietic elements in the hematopoietic systems of mice.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(610)

**Type:** other: Hematotoxicity

**Remark:** In the mouse, the concurrent evaluation of micronuclei frequencies in peripheral blood polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) permits an assessment of both recently-induced and chronically-accumulated bone-marrow damage. This assay system was used to evaluate on a weekly basis the effect of exposure duration (1-13 weeks, 6 h per day) and exposure regimen (Regimen 1:5 exposure days per week; Regimen 2:3 exposure days per week) on the ability of 300 ppm benzene to induce genotoxic damage in the bone marrow of male and female DBA/2 mice. In addition, an analysis of the percentage of PCE in peripheral blood was used to evaluate benzene-induced alterations in the rate of erythropoiesis. Exposure to benzene induced a marked increase in the frequency of micronucleated PCE (MN-PCE), an effect which was considerably greater in male mice than in female mice. In both sexes, the induction of MN-PCE was independent of exposure regimen and of exposure duration. Exposure to benzene also resulted in an exposure duration-dependent increase in the frequency of MN-NCE. The frequency of MN-NCE increased more slowly in female than in male mice and, within each sex, more slowly in Regimen 2 animals. Apparent steady-state conditions for MN-NCE frequencies were attained by about the fifth week of exposure in female mice exposed by either regimen and in male mice exposed by Regimen 2. An analysis of % PCE data revealed an initial severe depression in the rate of erythropoiesis in both sexes, with the return in the production of PCE to control levels being dependent on both sex and exposure regimen. Suppression of PCE production occurred throughout the course of the study in Regimen 2 males, while the percentage of PCE returned to control levels sporadically after 5 weeks in Regimen 1 males and within 5 weeks in females, regardless of regimen. Thus, while the sex-dependent induction of genotoxic damage by multiple exposures to benzene over a 13-week period was independent of exposure regimen and duration, the induction of cytotoxic damage was both sex- and regimen-dependent. The most severe depression of erythropoiesis occurred in male DBA/2 mice exposed to benzene by the more intermittent regimen.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(707)

**Type:** other: Hematotoxicity

**Remark:** The effect of exposure duration and regimen on benzene induced-bone marrow damage was evaluated using B6C3F1 and C57B1/6 male mice. An analysis of peripheral blood smears taken weekly from these mice exposed to 300 ppm benzene for 13 weeks (6 h per day) for either 5 days per week (Regimen 1) or for 3 days per week (Regimen 2) revealed: (i) a highly significant increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCE), the magnitude of which was strain specific (DBA/2 > C57B1/6 = B6C3F1), but independent of exposure regimen and of exposure duration. In male B6C3F1 mice, MN-PCE frequencies increased slightly with increasing exposure duration: (ii) a strain- (C57B1/6 = B6C3F1 > DBA/2) and regimen- (Regimen 1 > Regimen 2) dependent increase across time in the frequency of micronucleated normochromatic erythrocytes (MN-NCE). Apparent steady-state conditions for MN-NCE frequencies were attained by about 5 weeks of exposure in male mice of all three strains exposed to benzene by Regimen 2. Steady-state conditions for MN-NCE frequencies in male mice exposed to benzene by Regimen 1 did not occur during the duration of the study, with strain-dependent differences in the kinetics of MN-NCE accumulation being present: and (iii) in all 3 strains, and initial severe depression in the rate of erythropoiesis, the return of which to normal levels was both strain- (C57B1/6 = B6C3F1 > DBA/2) and regimen- (Regimen 1 > Regimen 2) dependent. These data indicate that the induction of genotoxic and cytotoxic damage in the bone marrow of male mice exposed to benzene for 13 weeks can be highly dependent on strain, exposure regimen and exposure duration but that under no circumstance did the level of genotoxic damage induced by benzene decrease under multiple exposure conditions.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(706)

**Type:** other: Hematotoxicity

**Remark:** An in vitro assay of a primitive hematopoietic precursor (high proliferative potential colony-forming unit, CFU-HPP), was used to investigate damage to the stem cell compartment in male B6C3F1 mice exposed to 0, 1, 10, 100 or 200 ppm benzene (6 hr/day, 5 days/week) by inhalation for 1, 2, 4 or 8 weeks. Six or 7 mice were exposed at each dose and time point. Exposure to 1 ppm had no significant effect on femoral cellularity, the number of CFU-HPP/femur or the number of CFU-HPP/5x10<sup>4</sup> marrow cells. Exposure to 10 ppm had no significant effect on femoral cellularity but did cause a significant reduction in the number of cfu-Hpp/5x10<sup>4</sup> marrow cells at the 2-week time point, which returned to control values at 4 weeks. Exposure to 100 and 200 ppm significantly reduced femoral cellularity at 1, 2, 4 and 8 weeks. A significant reduction in the number of

CFU-HPP/femur was observed at 1, 2, 4 and 8 weeks (200 ppm) and 2, 4 and 8 weeks (100 ppm), and in the number of CFU-HHP/5x10(4) marrow cells at 2, 4 and 8 weeks (100 and 200 ppm). An investigation of recovery of the CFU-HPP population in mice following a 4-week exposure demonstrated that the number of CFU-HPP/femur and number of CFU-HPP/5x10(4) marrow cells approach control levels within 11 (100 ppm) and 18 (200 ppm) days.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(951)

**Type:** other: Hematotoxicity

**Remark:** The hematopoietic cell response to benzene intoxication in mice (during and after long-term inhalation) was analyzed by a mathematical model of murine hematopoiesis. Two complementary methods, Time-Curve and Steady-State Analysis, were developed to identify target cells for benzene toxicity and to quantify the extent of damage in different stages of development of these target cells. Erythropoietic cells were the most sensitive; (ii) granulopoietic cells were about half as sensitive as erythropoietic and (iii) hematopoietic stem cells exhibited a sensitivity that ranged between that of erythropoietic and granulopoietic cells. A dose-response relationship between benzene levels and damage in target cells (valid from 1 to more than 900 ppm) was derived that was linear for doses up to 300 ppm and plateaued thereafter. This relationship indicated that benzene-induced hematotoxicity is subject to a saturable process. Recovery of hematopoiesis following chronic benzene intoxication was simulated for different doses and preceding exposure periods. The impaired recovery following exposure periods > 8 weeks could be explained by a severe reduction in the maximum self-maintenance of stem cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1012)

**Type:** other: Hematotoxicity

**Remark:** Using the <sup>59</sup>Fe uptake method of Lee et al. it was shown that erythropoiesis in female mice was inhibited following IP administration of benzene, hydroquinone, p-benzoquinone, and muconaldehyde. Toluene protected against the effects of benzene. Coadministration of phenol plus either hydroquinone or catechol resulted in greatly increased toxicity. The combination of metabolites most effective in reducing iron uptake was hydroquinone plus muconaldehyde. We have also shown that treating animals with benzene leads to the formation of adducts of bone marrow DNA as measured by the <sup>32</sup>P-postlabeling technique.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1078)

**Type:** other: Hematotoxicity

**Remark:** Studies of experimental animals following acute or chronic exposure to benzene by a variety of routes indicate that benzene exerts its effects on early blood cell precursors such as the pronormoblast, normoblast and promyelocyte. Recent studies with benzene strongly indicate that the metabolite(s) of benzene mediate its hemopoietic toxicity. Inhibition of benzene metabolism by competitive inhibitors or by partial hepatectomy protects against benzene toxicity. Although the central role of the liver in benzene metabolism has been recognized for many years, recent experiments demonstrate that bone marrow itself can metabolize benzene, but at a much slower rate than the liver. Benzene is probably first metabolized by liver to a metabolite that then undergoes further activation in bone marrow. Experimental evidence suggests that the electrophilic species that binds to both liver and bone marrow protein is not benzene epoxide as previously thought but a microsomal oxidation product of phenol. Pyrocatecholor hydroquinone are other phenolic metabolites of benzene that may be further activated to electrophilic species that then bind to protein or DNA.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1077)

**Type:** other: Hematotoxicity/Immunotoxicity

**Remark:** This study analyzed the effects of benzene on the morphology and functional activity of bone marrow phagocytes. Male Balb/c mice were treated with benzene (660 mg/kg) once per day for 3 days. Bone marrow cells were then isolated and fractionated by density gradient centrifugation. Using flow cytometry/cell sorting, three distinct populations of bone marrow cells that differed with respect to size and density could be separated. Monoclonal antibody binding and cell sorting revealed a large, dense population that consisted predominantly of granulocytes, a smaller, less dense population of lymphocytes, and a population of intermediate size and density consisting of mononuclear phagocytes and precursor cells. Differential staining of sorted mononuclear phagocytes revealed that benzene treatment of mice caused a marked increase in the number of mature, morphologically activated macrophages in the bone marrow. Benzene treatment of mice also resulted in enhanced chemotaxis and production of hydrogen peroxide by bone marrow granulocytes and mononuclear phagocytes. In contrast, treatment of mice with the combination of hydroquinone and phenol (50 mg/kg each, 1x/day, 3 days), two metabolites of benzene, resulted in a significant depression of granulocyte chemotaxis and had no effect on hydrogen peroxide production by bone marrow phagocytes compared to cells from control animals. Taken together these results demonstrate that benzene causes increased differentiation and/or activation of phagocytes in the bone marrow.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(716)

**Type:** other: Hematotoxicity/Immunotoxicity

**Remark:** Leukemogenesis is recognized to be a multifactorial process involving more than a single direct cytotoxic effect on a cycling progenitor cell. In this paper, the authors hypothesize that a distinguishing characteristic of agents with leukemogenic potential is their ability to produce intrinsic alterations in the regulation of stem cell differentiation in addition to targeting dividing cells. Benzene metabolism is a requirement for toxicity, and the phenolic metabolites of benzene have been repeatedly implicated in hematotoxicity. In this paper the authors studied the effects of in vitro pretreatment with benzene metabolites on colony-forming response of murine bone marrow cells stimulated with recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF). Pretreatment with hydroquinone (HQ) at concentrations ranging from picomolar to micromolar for 30 minutes resulted in a 1.5 to 4.6-fold enhancement in colonies formed in response to rGM-CSF that was due to an increase in granulocyte/macrophage colonies. Optimal enhancement was obtained with 1 micromolar HQ and was largely independent of the concentration of rGM-CSF. Pretreatment with other authentic benzene metabolites, phenol and catechol, and the putative metabolite trans,trans-muconaldehyde did not enhance growth factor response. Coadministration of phenol and HQ did not enhance the maximal rGM-CSF response obtained with HQ alone but shifted the optimal concentration to 100 picomolar. The mechanisms underlying the HQ-induced enhanced response to GM-SCF need to be further evaluated.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(567)

**Type:** other: Immunotoxicity/Metabolism

**Remark:** The stromal macrophage, a target of benzene (BZ) toxicity, is involved in hematopoietic regulation through synthesis of several cytokines, including interleukin-1 (IL-1), which synergizes with IL-3 to promote development of the pluripotent stem cell to myeloid and lymphoid stem cells. Monocyte dysfunction and decreased IL-1 production have been shown to be involved in aplastic anemia in humans. This study demonstrated that hydroquinone (HQ), a toxic bone marrow (BM) metabolite of BZ produced a time- and concentration-dependent inhibition of the processing of the 34-Kd pre-interleukin-1 alpha (IL-1 alpha) to the 17-Kd mature cytokine in murine P388D1 macrophages and BM stromal macrophages, as measured by Western immunoblots of cell lysate proteins using a polyclonal rabbit anti-murine IL-1 alpha antibody. HQ over a 10-fold concentration range had no effect on the lipopolysaccharide (LPS)-induced production of pre-IL-1 alpha precursor or on cell viability or DNA and protein synthesis. Stromal macrophages obtained from the femoral BM of C57B1/6 mice exposed to BZ (600 or

800 mg/kg) body weight for 2 days were incapable of processing the 34-KD pre-IL-1 alpha to the mature 17-Kd cytokine when stimulated in culture with LPS. Stromal macrophages from mice coadministered BZ and indomethacin, a prostaglandin H synthase (PHS) inhibitor that has been shown to prevent BZ-induced myelotoxic and genotoxic effects in mice when coadministered with benzene were able to convert the pre-IL-1 alpha to mature cytokine. Administration of recombinant murine IL-1 alpha to mice before a dose of BZ that causes severe depression of BM cellularity completely prevents BM depression, most probably by bypassing the inability of the stromal macrophage in BZ-treated animals to process pre-IL-1 alpha to the mature cytokine.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(933)

**Type:** other: Leukemogenesis

**Remark:** Leukemias are monoclonal tumors that arise from cells in the hematopoietic stem and progenitor cell compartment. Consistent with emerging models of carcinogenesis, leukemogenesis is an evolutionary process involving multiple independent genetic and epigenetic events. Leukemia can develop secondary to alkylating drug therapy or exposure to benzene in which progressive dysplastic changes, accompanied by a distinct pattern of clonal cytogenetic abnormalities, give rise to acute myelogenous leukemia. Characterization of these clonal chromosomal aberrations together with observed alterations in other growth-promoting genes, provides a useful framework for studying chemical leukemogenesis and for use in understanding the origins and development of leukemia in general.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(571)

**Type:** other: Leukemogenesis

**Remark:** Interleukin-3 (IL-3) and granulocyte/macrophage colony stimulating factor (GM-CSF) are responsible for maintaining survival and stimulating growth of early dormant hematopoietic progenitor cells (HPC). Previous studies have revealed that pretreatment of murine HPC with hydroquinone (HQ) but not phenol, catechol or trans,trans-muconaldehyde, results in a selective enhancement of GM-CSF, but not IL-3-mediated clonogenic response. HQ pretreatment of murine HPC did not induce either an up- or a down-regulation of GM-CSF-receptors or any change in receptor affinity. CD34+ cells, which represent between 1 and 5% of human bone marrow, contain virtually all clonogenic stem and HPC. Pretreatment of CD34+ cells with HQ also results in enhanced clonogenic response with GM-CSF but not IL-3. These findings suggest that an early step in chemical leukemogenesis may involve transient alterations in the regulation of cytokine response to GM-CSF.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(570)

**Type:** other: Mechanisms

**Remark:** Benzene causes bone marrow depression leading to aplastic anemia in animals and humans and also induces acute myelogenous leukemia in humans. In addition to chromosome aberrations, sister chromatid exchange, and micronucleus formation, chromosomal translocations are significant. The mutagenic activity of benzene metabolites is placed in perspective. A pathway from exposure to benzene to eventualeukemia is discussed in terms of biochemical mechanisms, the role of cytokines and related factors, latency, and expression of leukemia.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1073)

**Type:** other: Metabolism

**Remark:** The effect of acute, high-dose exposure to benzene on hepatic bioactivation (CYP2E1) and detoxication (glutathione transferase and aldehyde dehydrogenase) enzymes has been defined, while little is known about the effect of repeated, low dose benzene exposure on these enzymes. Female CD-1 mice were treated by gavage for 3 wk with benzene doses of 5 mg/kg or 50 mg/kg in corn oil. These doses of benzene produced 0.048 and 0.236 micromol muconic acid/d, respectively. The authors found that repeated administration to 50 mg benzene/kg/d decreased CYP2E1 activity by 34% and induced glutathione transferase activity by 30% without affecting aldehyde dehydrogenase activity.

**Source:** German rapporteur**Flag:** Risk Assessment

08-JUL-2005

(270)

**Type:** other: Metabolism

**Remark:** The pathways for benzene oxidative metabolism are generally understood and involve the cytochrome P-450 family of enzymes. Transgenic CYP2E1 knockout mice were used to investigate the involvement of CYP2E1 in the in vivo metabolism of benzene and in the development of benzene-induced toxicity. For the metabolism studies, male transgenic and wild-type control mice were exposed to 200 ppm benzene, along with a radiolabeled tracer dose <sup>14</sup>C-benzene by nose-only inhalation for 6 hr. Total urinary radioactivity and all radiolabeled individual metabolites were reduced in urine of transgenic mice compared to wild-type controls during the 48-hr period after benzene exposure. In addition, a significant greater percentage of total urinary radioactivity could be accounted for as phenylsulfate conjugates in transgenic mice compared to wild-type mice, indicating the importance of Cyp2E1 in oxidation of phenol following benzene exposure in normal mice. Because benzene oxidative metabolism was not

completely abolished in transgenic CYP2E1 knockout mice, metabolism must occur through other metabolic enzymes. Hepatic CYP2B1 metabolizes benzene in rat liver microsomes, although at much higher concentrations than those needed for catalysis by CYP2E1. It is likely that this enzyme has a significant contribution in the oxidative metabolism of benzene in vivo in transgenic CYP2E1 knockout mice. For the toxicity studies, male transgenic CYP2E1 knockout, wild-type, and B6C3F1 mice were exposed by whole-body inhalation to 0 ppm or 200 ppm benzene, 6 hr/day for 5 days. On day 5, blood, bone marrow, thymus, and spleen were removed for evaluation of micronuclei frequencies and tissue cellularities. No benzene-induced cytotoxicity or genotoxicity was observed in transgenic CYP2E1 knockout mice. In contrast, benzene exposure resulted in severe genotoxicity and cytotoxicity in both wild-type and B6C3F1 mice. These studies conclusively demonstrate that CYP2E1 is the major determinant of in vivo benzene metabolism and benzene-induced myelotoxicity in mice.

**Source:** German rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(1198)

**Type:** other: Modeling

**Remark:** The issue of parametric variability in a physiological model of benzene pharmacokinetics is addressed. Monte Carlo simulations were used to study the effects on the model output arising from variability in its parameters. The output was classified into two categories, depending on whether the output of the model on a particular run was judged to be generally consistent with published experimental data. Statistical techniques were used to examine sensitivity and interaction in the parameter space. The model was evaluated against the data from three different experiments in order to test for the structural adequacy of the model and the consistency of the experimental results. The regions of the parameter space associated with various inhalation and gavage experiments are distinct, and the model as presently structured cannot adequately represent the outcomes of all experiments.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(1081)

**Type:** other: Modeling

**Remark:** This study was conducted to determine whether formation of benzene-derived adducts with blood hemoglobin (Hb) can be used as a biomarker of exposure to benzene. B6C3F1 mice and F344/N rats were given 0.1 to 10,000  $\mu\text{mol}$  [ $^{14}\text{C}$ ]benzene/kg body wt. orally. Twenty-four hours later, animals were euthanized, and globin was isolated from blood samples. The globin was analyzed by liquid scintillation spectrometry for the presence of [ $^{14}\text{C}$ ]benzene-derived adducts. Hb adduct

formation was linear with respect to dose for amounts of up to 500 umol [14C]benzene/kg body wt. for both rodent species. Within this linear dose-response range, mice formed adducts from [14C]benzene approximately 3.5 times less efficiently than did rats. Benzene-derived Hb adducts also accumulated linearly when mice and rats were given up to three daily doses of 500 umol [14C]benzene/kg body wt. These data were used to develop a physiological model for benzene-derived Hb adduct formation. Both first-order and saturable pathways for adduct formation were incorporated. The results showed that the model simulated the levels of Hb adducts in both mice and rats after oral exposures to benzene and predicted the levels of Hb adducts present after inhalation exposure. These studies suggest that Hb adducts might be useful biomarkers for human exposures to benzene.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1099)

**Type:** other: Modeling

**Remark:** A physiologically based pharmacokinetic model was developed and used to describe the pharmacokinetics of benzene in three species: mice, rats, and humans. For each species, the body was divided into five anatomical compartments, consisting of liver, fat, bone marrow, and muscle, and organs such as brain, heart, kidney, and viscera, connected by the arterial and venous blood flow pathways. Metabolism of benzene followed Michaelis-Menten (nonlinear) kinetics in all species and occurred primarily in the liver compartment and, to a lesser extent, in the bone marrow. Comparison of model results with empirical data on inhalation, gavage, and intraperitoneal and subcutaneous injection in mice, rats, and humans, demonstrated the utility of a physiological pharmacokinetic model in describing the pharmacokinetics of benzene in three species across multiple routes of exposure.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(1154)

**Type:** other: Modelling

**Remark:** This paper examines the animal-based risk assessments for benzene using physiologically-based pharmacokinetic (PBPK) models of benzene metabolism in animals and humans. It demonstrates that internal doses (interpreted as total benzene metabolites formed) from oral gavage experiments in mice are well predicted by a PBPK model developed by Travis et al. The PBPK modeling validates the use of nonlinear regression models, previously used by Bailer and Hoel. An important finding is that refitting the linearized multistage (LMS) model family to internal doses and observed responses changes the maximum-likelihood estimate (MLE) dose-response curve for mice from linear-quadratic to

cubic, leading to low-dose risk estimates smaller than in previous risk assessments. Estimates of human risks at low doses are reduced by the use of internal dose estimates when the estimates are obtained from a PBPK model.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** German Rapporteur  
Risk Assessment

07-JUL-2005

(254)

**Type:** other: Modelling

**Remark:** Use of Monte-Carlo uncertainty analysis and physiologically-based pharmacokinetic (PBPK) models were used to quantify internal dose-response and to extrapolate risk from mice to humans for benzene. The analysis found that there is no evidence of a positive relation between benzene exposure and cancer probability at benzene concentrations below 1 ppm. The models suggest that the curve relating benzene concentration to AML risk at sufficiently low, constant concentrations of benzene approaches zero at dose levels below 10 ppm. The evaluation concluded that, for the same total administered dose, higher concentrations of benzene may be more hematotoxic.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur  
**Flag:** Risk Assessment

14-SEP-2000

(250)

**Type:** other: Modelling

**Remark:** This paper examines the animal-based benzene cancer risk assessments using physiologically-based pharmacokinetic (PBPK) models of benzene metabolism in animals and humans. Internal doses (total benzene metabolites) from oral gavage experiments in mice are well-described by a simple nonlinear (Michaelis-Menten) regression model. Refitting the multistage model family to internal doses changes the maximum-likelihood estimate (MLE) dose-response curve for mice from linear-quadratic to purely cubic, so that low-dose risk estimates are smaller than in previous risk assessments. The use of internal dose estimates for humans from a PBPK model reduces estimated human risks at low doses. A Monte-Carlo uncertainty analysis approach suggests that the excess risk due to benzene exposure may be non-existent at sufficiently low doses. Two types of biological information about benzene effects -- pharmacokinetic and hematotoxic -- are examined to test the plausibility of this finding. A framework for incorporating causally relevant biological information into benzene risk assessment is introduced, and it is shown that both pharmacokinetic and hematotoxic models appear to be consistent with the hypothesis that sufficiently low concentrations of inhaled benzene do not create an excess risk.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(253)

**Type:** other: Modelling

**Remark:** Study reports on the use of physiologically-based pharmacokinetic (PBPK) models to evaluate benzene metabolism in humans. The data indicate that the maximum rate of bone marrow metabolism reached for a given total cumulative exposure, is sensitive to the time pattern of administration. Exposures of relatively short periods to high concentrations of benzene tend to maximize internal concentrations of metabolites. Therefore, cumulative exposure is a good predictor for total amounts of benzene metabolized, but not of maximum internal concentrations.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(252)

**Type:** other: Modelling

**Remark:** A physiological model was developed to describe the uptake and metabolism of benzene in rats and mice and to determine if the observed difference in toxic effects could be explained by differences in the pathways for metabolism of benzene or by differences in uptake of benzene. Major pathways for elimination of benzene included metabolism to hydroquinone glucuronide or hydroquinone sulfate, phenyl glucuronide or phenyl sulfate, muconic acid, and prephenyl mercapturic acid or phenyl mercapturic acid. Model simulations for total benzene metabolized and for profiles of benzene metabolites were conducted for oral or inhalation exposure and compared to data for urinary excretion of benzene metabolites after exposure of rats and mice to [<sup>14</sup>C]- or [<sup>3</sup>H]-benzene by inhalation or gavage. Results for total amount of benzene metabolized, expressed per kilogram body weight, indicated that for inhalation exposure concentrations up to 1000 ppm, mice metabolized at least two to three times as much benzene as did rats. Simulations of oral exposure to benzene resulted in more benzene metabolized per kilogram body weight by rats at oral exposures of greater than 50 mg/kg. Patterns of metabolites formed after either route of exposure were very different for rats and mice. Rats primarily formed the detoxification metabolite, phenyl sulfate. Mice formed hydroquinone glucuronide and muconic acid in addition to phenyl sulfate. Hydroquinone and muconic acid are associated with pathways leading to the formation of the putative toxic metabolites of benzene. Metabolic rate parameters, were very different for hydroquinone conjugate and muconic acid formation compared to formation of phenyl conjugates and phenyl mercapturic acids. Putative toxication pathways could be characterized as high affinity, low capacity whereas detoxification pathways were low affinity, high capacity. Model simulations suggested that for both rats and mice at

lower exposure concentrations hydroquinone and muconic acid represented a larger fraction of the total benzene metabolized than at higher exposure concentrations where detoxification metabolites were predominant.

**Source:** Deutsche Shell Chemie GmbH Eschborn ;German rapporteur

**Flag:** Risk Assessment

14-SEP-2000

(761)

**Type:** other: PBPK model

**Remark:** The Medinsky benzene PBPK model was based on four compartments (liver, poorly perfused tissues, richly perfused tissues, fat). It assumes that the liver is the only organ where metabolism takes place which is an obvious disadvantage of the model. The model was developed with physiologic, biochemical and partition coefficients from rats and mice. Human metabolic rate constants were derived by using the metabolic parameters for mice, the more sensitive rodent species, and simulating 8-hour inhalation exposure over a range of concentrations. Model simulation results were compared to experimentally derived values for total metabolism of benzene. A major limitation of the model is its lack of inclusion of bone marrow activation of phenolic metabolites by myeloperoxidase-catalyzed reactions under formation of muconaldehyde as a direct alkylating agent which rapidly reacts with GSH and other cellular nucleophiles.

**Source:** German Rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(760)

**Type:** other: Patient cytogenetics

**Remark:** Two patients with presumably benzene-induced malignant blood disorders with preleukemic phases were cytogenetically monitored through the courses of their diseases. Exposures were based on use of gasoline and glues containing benzene. Patient 1, in addition to a familial chromosome translocation [t(3:16)], developed karyotypic abnormalities in 100% of the marrow cells, including two translocations: t(9:10) and t(4:15). Monosomy of chromosome 7 characterized the cells of patient 2. Cytogenetic monitoring of the patients at various phases of their diseases served as an important indicator of the transformation or progression of the pre-leukemia into frank leukemia and of the unusual behavior of such leukemic cells.

**Source:** Deutsche Shell Chemie GmbH Eschborn

06-JAN-1997

(1201)

**Type:** other: Repeated Dose Toxicity

**Remark:** A leukoagglutination test and a cytotoxic test with leukocytes were performed on workers exposed to occupational contact with benzene, toluene and xylene. In the first group the presence of leukocyte agglutinins for autologous leukocytes was found in only one person and in the second group in 10 persons. The increase of leukoagglutination titer of sera after their incubation with benzene, toluene or xylene was also demonstrated. This suggested the occurrence of allergic blood dyscrasia in some persons exposed to benzene and its homologues.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
German Rapporteur

**Flag:** Risk Assessment

08-JUL-2005

(660)

**Type:** other: Review

**Remark:** Benzene causes leukemia in humans and a variety of solid tumors in rats and mice. Research on benzene metabolism, pharmacokinetics, cytotoxicity, genotoxicity, and carcinogenicity in vivo and in vitro are starting to converge on a small set of overlapping hypotheses about the most probable biological mechanisms of benzene toxicity and carcinogenicity. It seems likely that the ultimate answer to how benzene exerts its multiple effects will consist of elaborations and extensions of one or more of the current hypotheses. This paper reviews benzene health effects and biology, showing how various aspects of metabolism and cytotoxicity fit together with genotoxic and nongenotoxic effects to help explain how benzene may cause cancer.

**Source:** Deutsche Shell Chemie GmbH Eschborn

08-JUL-2005

(251)

**Type:** other: Strain differences in the covalent binding of benzene to macromolecules

**Remark:** B6C3F1 mice are more sensitive to the tumor-inducing effects of benzene than C57BL/6 mice. We compared the kinetics of (14C)benzene binding to macromolecules at a 5 ug/kg bw dose over 48 hrs in both mouse strains to determine if tumor sensitivity is metabolism related at low dose. Liver protein and DNA adducts reached maximal levels in both strains within 0.5-1 hr. Bone marrow DNA and protein adducts reached maximal levels 12-24 hr post-exposure in both strains and declined gradually thereafter. Protein binding in liver and bone marrow was 15-times greater than DNA binding in both strains and protein binding was greater in liver relative to bone marrow. At all time points, all adducts levels were ~ 2-4 times greater in B6C3F1 relative to C57BL/6 mice. The adduct formation and clearance kinetics suggests two distinct macromolecule binding phases in both strains possibly due to two metabolite pools. These data show that at low doses greater levels of bioactive

benzene metabolites are present in the liver and bone marrow of B6C3F1 than C57BL/6 mice and are consistent with the hypothesis that metabolism may contribute to the difference in benzene's carcinogenicity among these strains. This work was performed under the auspices of the US DOE (W7405-ENG-48) with support from HEI (94-5).

**Source:** EXXON Biomedical Sciences East Millstone, NJ  
**Reliability:** (4) not assignable

04-NOV-1998

(740)

**Remark:** methods: effects of benzene on murine resistance to an infectious agent (bacteria), *Listeria monocytogenes*, studied by exposure of 5-7 male C57Bl/6J mice/group to 0, 10, 31, 100, or 298 ppm (analytical concentration) for 6 h/d for a) 5 d prior to infection or b) 5 d prior to infection plus 7 d during infection.

results: significantly increased number of bacteria on day 4 of infection at 300 ppm in a) and in b) at all doses except the lowest; B and T lymphocytes significantly reduced at  $\geq$  31 ppm in a) and b);

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** no data

27-SEP-2000

(958)

**Remark:** method: 5 male CD-1 mice/group were exposed to 0, 31, 166 or 790 mg/l in the drinking water for 28 d (see also chapter 5.4); mice sensitized by i.p. injection with sheep red blood cell suspension on day 24 were sacrificed on day 28 and spleen lymphocytes cultered for further examinations.

results: see also chapter 5.4; lymphocyte proliferation to both B and T cell mitogens was enhanced at the low dose and depressed in the mid- and high-dosed group; similar biphasic effects with cell-mediated immunity to allogeneic cells and with cytotoxic T lymphocyte activity to YAC-1 tumor cells; antibody production as assessed by enumeration of the sheep cell-specific plaque forming cells indicated a significant suppression at 166 and 790 ppm.

**Source:** Deutsche Shell Chemie GmbH Eschborn  
**Test substance:** purity 99.9%

27-SEP-2000

(534)

**Remark:** methods: at least 5 male C57Bl mice per group were exposed to 0, 10, 31, 100 or 300 ppm 6 h/d for 6 d.

results: even exposure to 10 ppm resulted in significant depression in femoral lipopolysaccharide-induced B-colony-forming ability, while total number of B-lymphocytes were not significantly depressed; splenic phytohemagglutinin- induced blastogenesis was significantly depressed at 31 ppm without a depression in numbers of T-lymphocytes.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** no data

27-SEP-2000

(972)

**Remark:** method: groups of 10 male C57Bl/6J mice were exposed to 300 ppm for 6, 30, 115 exposures (6 h/day, 5 d/week).  
results: number of B-lymphocytes in bone marrow and spleen and number of T-lymphocytes in thymus and spleen significantly reduced after all 3 periods; progressive depression of mitogen-induced proliferation of bone marrow and splenic B-lymphocytes, also splenic T-cell mitogen-induced proliferation depressed but not progressively; marked increase in thymic and bone marrow cellularity.

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** no data

27-SEP-2000

(971)

**Remark:** methods: 15 female Swiss mice per group were exposed to 50, 200, or 400 ppm (6 h/d, 5 d per week) for 5, 12, or 22 exposures; after these exposure periods mice were immunized with 2 different tetanus toxoids.  
results: no effects at 50 ppm; inhibited primary antibody formation after 10 or 20 exposures to 200 ppm; 400 ppm increasingly suppressed primary antitoxin response to both antigens;

**Source:** Deutsche Shell Chemie GmbH Eschborn

**Test substance:** no data

27-SEP-2000

(1092)

**Remark:** Effects of benzene inhalation on T and B lymphocytes and immune responses in mice were examined. Male mice were exposed to 50 or 200 ppm benzene vapor, 6 hr/day for 7 or 14 consecutive days. T and B lymphocytes, in blood and spleen, were detected by the cytotoxicity assay and the membrane immunofluorescence test. Humoral immune response to sheep red blood cells was determined by the hemolytic plaque-forming cell assay. Cell-mediated immune response was measured by contact sensitivity (CS) to picryl chloride. The activity of suppressor cells was elevated in spleen by the suppressive effect on passive transfer of CS. The ratio and absolute number of T and B lymphocytes in blood and spleen were depressed after a 7-day exposure at 50 ppm benzene. The depression of B lymphocytes was dose dependent and more intense than that of T lymphocytes. The ability to form antibodies was suppressed by benzene at all exposure levels, but the CS response was resistant to benzene inhalation and rather enhanced at 200 ppm exposure for 14 days. The activity of suppressor cells could not be detected at this dose level. These data show that benzene inhalation effects on humoral and cell-mediated immune responses are a result of the selective toxicity of benzene

**Source:** to B lymphocytes and suppressor T cells.  
Deutsche Shell Chemie GmbH Eschborn

27-SEP-2000

(36)

**Remark:** Hydroquinone, a metabolite of benzene, inhibits microtubule assembly and suppresses lectin-induced transformation of lymphocytes. Other workers have established a role for microtubules in modulating cell response to blastogenic stimuli. Several lines of evidence suggest that a) suppression of lymphocyte activation by hydroquinone is mediated through interference with the cytoskeleton, specifically, inhibition of microtubule function, b) inactivation is an SH-dependent process, and c) p-benzoquinone, a direct metabolite of hydroquinone, is the most likely molecular species responsible for these events.

**Source:** Deutsche Shell Chemie GmbH Eschborn

27-SEP-2000

(573)

**Remark:** The sex difference in the susceptibility to haematopoietic disorders induced by benzene was studied kinetically with a special reference to its relation with the body fat content. In rats of both sexes with a large body fat content, benzene was eliminated more slowly and remained in the body for a longer time than in rats with a small body fat content. In accord with this finding, the decrease in whiteblood cell numbers during a chronic benzene exposure was observed only in the groups of rats which had a large volume of fat tissue. In an experimental human exposure, the elimination of benzene was slower in the females than in the males. The kinetic study revealed that the slower elimination in the females is due primarily to the bulky distribution of body fat tissue in that sex. From these results obtained from the experimental exposure of men and rats to benzene, it was concluded that the human female, with her body fat tissue, show an inherent disposition to be susceptible to a chemical such as benzene which has a high affinity with fat tissue.

**Source:** Deutsche Shell Chemie GmbH Eschborn ; German rapporteur  
**Flag:** Risk Assessment

27-SEP-2000

(1005)

**Remark:** The P450 isozyme composition of rabbit bone marrow was investigated. CYP2E1 and CYP1A1 were identified in the marrow and were found to be induced by treatments which induce them in the liver and in the bone marrow. CYP2E1 is the primary catalyst of benzene hydroxylation to phenol and hydroquinone. Its enzymic activity were determined for both hepatic and marrow microsomal preparations. Hepatic microsomes isolated from acetone-treated rabbits were 48 times more active than microsomes from untreated rabbits. Aroclor 1254 treatment did not significantly affect benzene hydroxylation. Acetone treatment resulted in a 9.4-fold

increase in the benzene hydroxylase activity of the marrow. In addition, the marrow from acetone-treated rabbits was the only preparation to give detectable levels of hydroquinone leading to the formation of phenol and hydroquinone in a ratio of about 7:1.

**Source:** German rapporteur  
**Flag:** Risk Assessment

28-JUN-2005

(1025)

**7.1 Function**

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**7.2 Effects on Organisms to be Controlled**

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**7.3 Organisms to be Protected**

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**7.4 User**

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**7.5 Resistance**

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