APPENDIX L

Potential Exposure to Ethylbenzene from Food-Contact Use of Polystyrene Resins (PWG 1997)

POTENTIAL EXPOSURE TO ETHYLBENZENE

FROM FOOD-CONTACT USE OF POLYSTYRENE RESINS

POLYSTYRENE WORK GROUP

FOOD, DRUG, AND COSMETIC PACKAGING MATERIALS COMMITTEE

SOCIETY OF THE PLASTICS INDUSTRY, INC.

DECEMBER 1997

Executive Summary

The Polystyrene Work Group of The Society of the Plastics Industry's (SPI) Food, Drug, and Cosmetic Packaging Materials Committee has estimated the maximum potential dietary exposure to ethylbenzene from its presence in food-contact polystyrene by employing published data on both the uses of polystyrene in food-contact applications and the potential migration behavior under the anticipated conditions of use. The total potential exposure to ethylbenzene from the food-contact use of polystyrene is approximately 0.45 ppb of an individual's diet, which corresponds to approximately 0.00135 mg/person/day, or alternatively, to approximately 0.0000225 mg/kg body weight/day.

I. Background

An industry-wide Polystyrene Work Group has been established from among the membership of The Society of the Plastics Industry, Inc. (SPI)[1/] to collect data on current food-contact applications of polystyrene. The Work Group has analyzed the relevant use data to determine the potential human dietary exposure to ethylbenzene as a result of its presence in polystyrene used in food-contact applications.

The estimate described below is based on the Food and Drug Administration's (FDA) general model for estimating exposure, *i.e.*, the postulated consumption of a substance used as a component of a food-contact material. This consumption depends (1) on the fraction of an individual's diet likely to contact packaging materials containing the substance and (2) on the potential level in food (*e.g.*, derived from migration values or predicted using mathematical modeling). In essence, the estimated dietary intake (EDI) is calculated by FDA by first multiplying the migration value of the substance (obtained from testing or calculations under the most severe time and temperature in actual use) by a consumption factor, which represents the portion of the total diet in contact with a given polymer or package used in a given application. Appropriate values to use for each of these two exposure-estimating elements are described below, followed by an estimate of exposure employing them.

1/ The Society of the Plastics Industry, Inc. is a trade association of nearly 2,000 members representing all segments of the plastics industry in the United States. SPI's business units and committees are composed of plastics processors, raw material suppliers, machinery manufacturers, moldmakers, and other industry-related groups and individuals. Founded in 1937, SPI serves as the "voice" of the plastics industry.

II. Polystyrene Use Patterns

FDA employs the term Consumption Factor (CF) to describe the portion of the diet likely to contact specific packaging materials. The CF is the ratio of the weight of food contacting a specific packaging material to the weight of all food packaged. FDA has established CF values for both packaging categories (e.g., metal, glass, polymer, and paper) and specific polymers.

FDA's current CF values are set forth in Appendix IV of the agency's "Recommendations for Chemistry Data for Indirect Food Additive Petitions," (June 1995). The agency has assigned a CF value of 0.1 (i.e., 10% of the diet) for "polystyrene." The polystyrene CF value has been increased slightly from the previous edition of the "Recommendations," issued in March, 1993; specifically, the polystyrene CF increased from 0.08 to 0.1.[2/] The agency clearly indicates in its "Recommendations," however, that it will rely on these values only as default values and is prepared to substitute alternative CF values where valid data are available that would justify the use of a more precise estimate.

It has always been important to use appropriate CF values to estimate possible exposure when presenting data to FDA in a food additive petition or another data file. However, it is even more critical to use CF values that are as well defined as possible for elucidating potential exposure to components of well-established plastics rather than rely on the default CF values, since the default values generally have been established based on the gross use of a given material and do not encompass refinements for specific uses. The use of the latter can permit reasonably accurate estimates of potential exposure to be calculated based on specific uses.

2/ It is important to recognize that, as noted in the 1995 "Recommendations," changes in the CF values, including that for polystyrene, were made to round the previous values up or down to achieve values having one decimal figure. Thus, this change does not reflect the acquisition of actual food-contact use data on any of the materials for which CF values have been modified, including polystyrene.

A refinement of the 0.08 polystyrene CF (*i.e.*, the CF in effect until June, 1995) has recently been published[3/] based on industry survey data on the uses of polystyrene in food-contact applications. The survey data in this publication were compared to published survey data on polystyrene.[4/] Based on these comparisons, we have concluded that the apportionment used to derive the refined polystyrene CF values is valid, since the market segments have remained relatively constant with regard to each other. However, because of the increase of the overall polystyrene CF value from 0.08 to 1.0 (in essence, an increase of 25%), we have "recalculated" the refined polystyrene CF values described in Lickly *et al.* by increasing them by 25%. These "updated" refined polystyrene values are described below in Tables 1 and 2.[5/]

3/ T.D. Lickly, C.V. Breder, and M.L. Rainey, "A Model for Estimating the Daily Dietary Intake of a Substance from Food-Contact Articles: Styrene from Polystyrene Food-Contact Polymers," Regulatory Toxicology and Pharmacology, **21**, 406-417, (1995). This report describes the method used to estimate potential exposure to styrene undertaken by the Ad Hoc Styrene Migration Task Group of SPI's Food, Drug, and Cosmetic Packaging Materials Committee (FDCPMC) and summarized in "The Safety of Styrene-Based Polymers for Food-Contact Use," issued on April 14, 1993.

4/ *Modern Plastics* Editors, "Key markets post solid gains in 1993," 75-76 (January 1994), *Modern Plastics* Editors, "A year of robust growth, rising costs," 65 (January 1995), *Modern Plastics* Editors, "Innovative technologies, expanding markets," 71 (January 1996), and *Modern Plastics* Editors, "Uniform properties for a common market," 77 (January 1997).

5/ The abbreviations used in Table 1 are as follows: GPPS = general purpose polystyrene; HIPS = high impact polystyrene; PS foam = polystyrene foam; EPS foam = expanded polystyrene foam.

Table 1. CFs for all applications of polystyrene packaging

Polymer/Applications	Refined CF
GPPS	
Produce Baskets	0.0011
Pie Containers	0.0011
Cookie Trays	0.0105
HIPS	
Yogurt Cups	0.0036
Cheese & Cream Containers	0.0036
Aseptic & Blow Molded Containers	0.0009
PS Foam	
Egg Cartons	0.0091
Stock Food Trays	0.0038
Total CF =	0.034

Table 2. CFs for all applications of polystyrene disposables

Polymer/Applications		Refined CF
GPPS (flatware & cutlery)		
Fatty:	75°F	0.0014
	130°F	0.0001
Aqueous:	75°F	0.0043
	130°F	0.0005
HIPS[6/]		
Fatty:	40°F	0.0001
	75°F	0.0001
	130°F	0.0003
Aqueous:	40°F	0.0108
	75°F	0.0188
	130°F	0.0016
Alcoholic		0.0015
PS Foam[7/]		
Fatty:	75°F	0.0030
	130°F	0.0031
Aqueous:	75°F	0.0060
	130°F	0.0070
EPS Foam[6/]		
Fatty:	75°F	0.0003
	130°F	0.0006
Aqueous:	75°F	0.0018
	130°F	0.0051
		Total CF = 0.066

6/ Tumblers, cocktail glasses, vending & portion cups, dishes, plates, bowls, lids, closures, and flatware.

7/ Cups, containers, single service plates, single service hinged containers, food service trays & other foam sheet.

III. Residual Ethylbenzene in Polystyrene

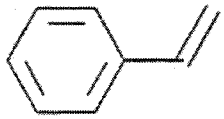
In addition to the information described above on the food-contact uses of polystyrene (as reflected in the refined CF values noted), the Polystyrene Work Group has conducted a survey of the industry to determine the levels of ethylbenzene in polystyrene in each of the polystyrene applications delineated in Tables 1 and 2. The weighted average levels of residual ethylbenzene in polystyrene are summarized in Table 3 below.

Table 3. Residual ethylbenzene levels for all applications of polystyrene packaging and disposables

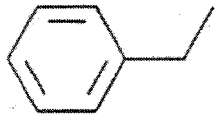
Polymer/Applications		Residual Ethylbenzene (ppm)
GPPS		
Produce Baskets		18
Pie Containers		18
Cookie Trays		18
HIPS		
Yogurt Cups		29
Cheese & Cream Containers		29
Aseptic & Blow Molded Containers		29
PS Foam		
Egg Cartons		66
Stock Food Trays		66
GPPS (disposables - flatware & cutlery)		
Fatty:	75°F	42
	130°F	42
Aqueous:	75°F	42
	130°F	42
HIPS (disposables)		
Fatty:	40°F	108
	75°F	108
	130°F	108
Aqueous:	40°F	108
	75°F	108
	130°F	108
Alcoholic		108
PS Foam (disposables)		
Fatty:	75°F	37
	130°F	37
Aqueous:	75°F	37
	130°F	37
EPS Foam (disposables)		
Fatty:	75°F	37
	130°F	37
Aqueous:	75°F	37
	130°F	37

IV. Considerations Relating to the Potential Migration of Ethylbenzene

Based on extensive studies on the migration of styrene from polystyrene food-contact articles, Lickly *et al.* concluded that the migration behavior of styrene may be predicted using Fickian diffusion theory. Because of the close structural similarity between styrene and ethylbenzene, as shown in Figure 1, we have similarly assumed that the migration behavior of ethylbenzene may be predicted using diffusion principles, although few studies on the migration of ethylbenzene have been published.



Styrene



Ethylbenzene

Figure 1. Structures of styrene and ethylbenzene

We summarize below the equations employed by Lickly *et al.* and described in detail therein. In instances where equilibrium partitioning may have an effect, such as with aqueous foods, migration may be generally estimated as

$$M_t = C_{p0}aK(1 - e^{Z^2}\,\mathrm{erfc}\,Z) \qquad (1)$$

where

$Z = (D_p t)^{1/2}/aK$
M_t = migration at time t in $\mu g/cm^2$
C_{p0} = initial residual concentration of substance in polymer in $\mu g/cm^3$
a = volume of food simulant in mL/cm^2
K = partition coefficient for substance between polymer and food simulant
D_p = diffusion coefficient of substance in the polymer
t = time of food contact in seconds
erfc = error function

In Lickly *et al.*, a simpler relationship than that expressed in equation (1) was employed, since the solution of the error function term ($1 - e^{Z^2}$ erfc Z) is a laborious procedure. Instead, the amount of migration into aqueous foods was determined as:

$$M_t = M_t^* C_{p0} aK \qquad (2)$$

where M_t^* is obtained by calculating Z (which is equal to $(D_p t)^{1/2}/aK$, as noted above) and determining M_t^* as described by Lickly *et al.*

Where equilibrium partitioning has little effect, such as with the migration of most organic substances into food oil or where there are very short exposure times to aqueous foods, equation (1) is reduced to:

$$M_t = 2C_{p0}(D_p t/\pi)^{1/2} \qquad (3)$$

Finally, in instances in which packaged food will experience two distinct temperatures during the overall time in which the food will be in the package, *e.g.*, where the packaged food will first be sterilized for a short period of time followed by long-term shelf storage, the total migration of the substance from both phases may be expressed as:

$$M_t = M_{t1} + M_{t2} \qquad (4)$$

where the subscripts 1 and 2 indicate first and second stages of temperature exposure.

The value of M_{t1} is calculated using equation (3) above, while the value of M_{t2} may be calculated as:

$$M_{t2} = 2C_{P01}(1/\pi)^{1/2}[(D_{p1}t_1 + D_{p2}t_2)^{1/2} - (D_{p1}t_1)^{1/2}] \quad (5)$$

Thus, the solutions to equations (2) through (6) require that values for t (time), C_{P01} (initial concentration of the substance), α (volume of food simulant), K (partition coefficient for the substance between polymer and food simulant), and D_p (diffusion coefficient of the substance in the polymer) be known. While values for t, C_{P01}, and α are defined by the use parameters, values for K and D_p must be determined for a given substance and polymer system.

In Lickly *et al.*, linear regression relationships of the diffusion coefficients (D_p) for styrene in GPPS, HIPS, and foamed polystyrene as a function of temperature (T, in degrees K) were determined from literature data on styrene migration as:

$$\text{GPPS:} \quad \log D_p = 2.724 - 4932(1/T) \quad (6)$$

$$\text{HIPS:} \quad \log D_p = 1.9407 - 4623.7(1/T) \quad (7)$$

$$\text{Foam:} \quad \log D_p = 4.543 - 4407.6(1/T) \quad (8)$$

In addition, a linear regression relationship between the partition coefficient (K) as a function of temperature (T) was determined from literature data as:

$$\log K = 2.2725 - 1773.9(1/T) \quad (9)$$

Using the appropriate equations noted above, Lickly *et al.* calculated the potential migration of styrene by employing the actual time and temperature use conditions for the known

applications of polystyrene, the estimated residual levels of styrene, and the diffusion and partition coefficients determined, as appropriate, using the equations described above.[8/] The time and temperature conditions and diffusion equations used for each application is summarized below in Table 4.

8/ The one exception to the use of calculations based on the principles of diffusion was that involving the use of polystyrene foam for egg cartons. In this instance, the amount of migration was based on the limit of detection of actual egg carton samples exposed to an aqueous simulant under the exposure conditions delineated in Lickly *et al.*

Table 4. Time and temperature conditions and diffusion coefficient equations for polystyrene packaging and disposables

Polymer/Applications	Time/Temperature	Diffusion Coefficient Equation
GPPS		
Produce Baskets	7 days/40°F	(2)
Pie Containers	30 days/40°F	(2)
Cookie Trays	60 days/75°F	(2)
HIPS		
Yogurt Cups	30 min/150°F +60 days/40°F	(4)
Cheese/Cream Containers	30 days/40°F	(2)
Aseptic Containers	60 days/75°F	(2)
PS Foam		
Egg Cartons	10 days/40°F	-
Stock Food Trays	30 days/40°F	(3)
GPPS (disposables)		
Fatty: 75°F	1 hr/75°F	(3)
130°F	1 hr/135°F	(3)
Aqueous: 75°F	1 hr/75°F	(3)
130°F	1 hr/135°F	(3)
HIPS (disposables)		
Fatty: 40°F	1 hr/40°F	(3)
75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)
Aqueous: 40°F	1 hr/40°F	(3)
75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)
Alcoholic	1 hr/75°F	(3)
PS Foam (disposables)		
Fatty: 75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)
Aqueous: 75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)
EPS Foam (disposables)		
Fatty: 75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)
Aqueous: 75°F	1 hr/75°F	(3)
130°F	1 hr/130°F	(3)

We have followed the procedure described by Lickly *et al* and summarized above to similarly calculate the potential exposure to ethylbenzene from the use of polystyrene in food-contact applications. However, several parameters used in the evaluation of ethylbenzene were different than those employed by Lickly *et al.* These are discussed below.

A. Diffusion Coefficients

The diffusion coefficients obtained using equations (6), (7), and (8) were "corrected" to account for the difference between styrene and ethylbenzene. Specifically, the general diffusive behavior of ethylbenzene is expected to be quite similar to that of styrene based on the close similarity in the structures of the two substances, as is evident from Figure 1. Therefore, we have concluded that the only difference in diffusion will arise because the molecular mass of ethylbenzene is slightly greater than that of styrene.

For insight into the comparative diffusion of styrene and ethylbenzene based on a difference in molecular weight, we have relied on literature modeling of diffusion. Specifically, we have turned to recent publications by Piringer *et al.* that present a model for estimating the diffusion of organic substances from plastic materials.[9/] The Piringer predictive model has the form:

$$D = 10^4 \exp(A_p - a\, M_r - b\, T^{-1}) \qquad (10)$$

where D is the diffusion coefficient, A_p accounts for the effect of the polymer on diffusivity, M_r is the substance's molecular weight, T is the temperature in degrees Kelvin, and *a* and *b* are

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9/ O.G. Piringer, "Evaluation of Plastics for Food Packaging," Food Additives and Contaminants, 1994, **11(2)**, 221-230; A.L. Baner, R. Franz, and O. Piringer, "Alternative Methods for the Determination and Evaluation of Migration Potential from Polymeric Food Contact Materials," Deutsche Lebensmittel-Rundschau, 1994, **90(5)**, 137-143 and **90(6)**, 181-185; A. Baner, J. Brandisch, R. Franz, and O.G. Piringer, "The applications of a predictive migration model for evaluating the compliance of plastic materials with European food regulations," Food Additives and Contaminants, 1996, **13(5)**, 587-601.

correlation constants for the effects on diffusion of the molecular weight and temperature, respectively. Values for *a* and *b* (0.01 and 10450, respectively) are independent of the polymer system. Values for several types of polymers have been derived, but a value for polystyrene has yet to be established. However, as demonstrated below, the absence of a specific A_p value for polystyrene will not preclude our use of equation (10) for comparative purposes.

From equation (10), the diffusion coefficient, D_1, for substance 1 having molecular weight M_{r1} through polymer X (with a polymer diffusivity value of A_{pX}) at temperature T_1 may be expressed as follows:

$$D_1 = 10^4 \exp(A_{pX} - a\, M_{r1} - b\, T_1^{-1}) \tag{11}$$

This equation can be expressed alternatively as:

$$D_1 = 10^4 \exp(A_{pX})/\exp(aM_{r1})\exp(bT_1^{-1}) \tag{12}$$

Likewise, the expression for substance 2, also through polymer X, would be:

$$D_2 = 10^4 \exp(A_{pX})/\exp(aM_{r2})\exp(bT_2^{-1}) \tag{13}$$

Dividing equation (14) by equation (13) gives:

$$\frac{D_2}{D_1} = \frac{10^4 \exp(A_{pX}) \exp(aM_{r1}) \exp(bT_1^{-1})}{10^4 \exp(A_{pX}) \exp(aM_{r2}) \exp(bT_2^{-1})} \tag{14}$$

For the case where the temperature for substance 1 and substance 2 are the same (*i.e.*, $T_1 = T_2$), equation (14) becomes:

$$\frac{D_2}{D_1} = \frac{\exp(aM_{r1})}{\exp(aM_{r2})} \tag{15}$$

or $D_2 = D_1 \exp(a(M_{r1}-M_{r2}))$. (16)

The molecular weight of styrene is 104 Daltons ($M_{r1} = 104$) and that of ethylbenzene is 106 Daltons ($M_{r2} = 106$). Thus, using equation (16), a diffusion coefficient for ethylbenzene may be obtained by "correcting" a styrene diffusion coefficient by a factor of 0.98, *i.e.*, $D_2 = 0.98D_1$, since $a = 0.01$.[10/]

In this way, ethylbenzene diffusion coefficients were calculated using equation (16), based on comparisons to calculations for styrene diffusion using equations (6), (7), and (8).

B. Partition Coefficients

We have the assumed that the relative solubilities of styrene and ethylbenzene in the polymers and in aqueous media are the same. Thus, we have determined the partition coefficients for ethylbenzene in the identical fashion as that employed by Lickly *et al.*

C. Modification for Mineral Oil Content

Lickly *et al* modified the calculated styrene migration levels for certain polystyrene applications due to the presence of mineral oil in the polymer. In these instances, the levels of mineral oil in polystyrene were assumed values rather than those that were measured. Specifically, the calculated migration levels of styrene from HIPS packaging (*i.e.*, used in yogurt cups, cheese and cream containers, and aseptic containers) were multiplied by a factor of 5 to account for the possibility that the articles could contain up to 3.5% mineral oil. Likewise, the styrene migration levels from GPPS disposables and HIPS disposables were multiplied by factors of 3.16 and 5, respectively, to account for the possibility that they could contain up to

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10/ In essence, the diffusion coefficients for styrene and ethylbenzene are virtually identical, since the difference between them is only a factor of 2%.

4.5% mineral oil and 3.5% mineral oil, respectively. The multiplication factors were determined from graphical data relating the mineral oil content of GPPS and HIPS to the apparent increase in their diffusion coefficients, based on the SPI report that preceded the publication by Lickly *et al.*[11/]

Since the publication by Lickly *et al.*, the Polystyrene Work Group of SPI's FDCPMC issued a report, entitled "Report on potential exposure to mineral oil from food-contact use of polystyrene resins," in March, 1996. Included in this report was an estimate of the mineral oil content of polystyrene based on a survey of the industry. The weighted average mineral oil content of HIPS packaging used in yogurt cups, cheese and cream containers, and aseptic containers were reported in the 1996 SPI report to be 2.5%, 2.8% and 2.9%, respectively. Likewise, the weighted average mineral oil content of GPPS disposables, and HIPS disposables reported in the 1996 SPI report were 1.3% and 2.5%, respectively. Using these more realistic values rather than the mineral oil levels assumed by Lickly *et al.*, we have used the same approach as Lickly *et al.* to determine multiplication factors to modify the calculated ethylbenzene migration levels for HIP packaging, GPPS disposables, and HIPS disposables.

Specifically, since the weighted average mineral oil content of HIPS packaging used in yogurt cups, cheese and cream containers, and aseptic containers were reported in the 1996 SPI report to be 2.5%, 2.8% and 2.9%, respectively, the multiplication factors used to modify the calculated migration levels for ethylbenzene for these applications, determined as described by Lickly *et al.*, were 3.6, 3.9, and 4.0, respectively. Likewise, because the weighted average mineral oil content of GPPS disposables, and HIPS disposables reported in the 1996 SPI report were 1.3% and 2.5%, respectively, the multiplication factors used to modify the calculated migration levels for ethylbenzene for these applications were 1.4 and 3.6, respectively.

11/ "The Safety of Styrene-Based Polymers for Food-Contact Use," issued on April 14, 1993 by the Ad Hoc Styrene Migration Task Group of SPI's Food, Drug, and Cosmetic Packaging Materials Committee (FDCPMC).

V. Estimate of Potential Exposure to Ethylbenzene

Based on the above considerations relating to the potential migration of ethylbenzene from polystyrene used in food-contact applications, the potential migration of migration for each of the applications described in Tables 1 and 2 were calculated. Furthermore, using the refined consumption factors (CF) summarized in Tables 1 and 2, estimates of potential dietary exposure to ethylbenzene were determined for each application. The results of these calculations are summarized in Table 5.

Table 5. Estimated ethylbenzene migration and exposure

Polymer/Applications		Migration (ppb)	Refined CF	Exposure (ppb)
GPPS				
Produce Baskets		0.273	0.0011	0.0003
Pie Containers		0.485	0.0011	0.0005
Cookie Trays		2.07	0.0105	0.0217
HIPS				
Yogurt Cups		9.15	0.0036	0.0329
Cheese/Cream Containers		3.05	0.0036	0.0110
Aseptic Container		13.3	0.0009	0.0120
PS Foam				
Egg Cartons		8	0.0038	0.0304
Stock Food Trays		18.6	0.0091	0.169
GPPS (disposables)				
Fatty:	75°F	0.283	0.0014	0.0004
	130°F	1.69	0.0001	0.0002
Aqueous:	75°F	0.283	0.0043	0.0012
	130°F	1.69	0.0005	0.0008
HIPS (disposables)				
Fatty:	40°F	0.772	0.0001	0.0001
	75°F	2.70	0.0001	0.0003
	130°F	14.4	0.0003	0.0043
Aqueous:	40°F	0.772	0.0108	0.0083
	75°F	2.70	0.0188	0.0508
	130°F	14.4	0.0016	0.0231
Alcoholic		2.70	0.0015	0.0041
PS Foam (disposables)				
Fatty:	75°F	0.951	0.0030	0.0029
	130°F	4.69	0.0031	0.0145
Aqueous:	75°F	0.951	0.0060	0.0057
	130°F	4.69	0.0070	0.0328
EPS Foam (disposables)				
Fatty:	75°F	0.688	0.0003	0.0002
	130°F	3.39	0.0006	0.0020
Aqueous:	75°F	0.688	0.0018	0.0012
	130°F	3.39	0.0051	0.0173
			Total =	0.45

VI. Summary

We have estimated the maximum potential dietary exposure to ethylbenzene from its presence in food-contact polystyrene by employing published data on both the uses of polystyrene in food-contact applications and the potential migration behavior under the anticipated conditions of use. The total potential exposure to ethylbenzene from all of these uses is approximately 0.45 ppb of an individual's diet, which corresponds to approximately 0.00135 mg/person/day, using FDA's assumption that an individual consumes 3000 grams of food (including fluids) each day. Further, using FDA's assumption that an individual weighs 60 kg, the total potential exposure to ethylbenzene from all of these uses corresponds to approximately 0.0000225 mg/kg body weight/day.

APPENDIX M

FDA Food Consumption Rates

U.S. Food and Drug Administration Total Diet Study Food Consumption Amounts (g/day)

TDS Diets, Version 2 (1990 food list + 1994-96, 1998 CSFII data)

OLD FOOD LIST 1990-2002

TDS food #	Food Description	Total US	6-11 mo. INCL addons	M/F 2 yr INCL addons	M/F 6 yr	M/F 10 yr	F 14-16 yr	M 14-16 yr	F 25-30 yr	M 25-30 yr	F 40-45 yr	M 40-45 yr
1	whole milk, fluid	64.75	83.72	180.25	139.01	108.69	62.97	103.14	44.61	57.09	26.79	53.52
2	low-fat milk, 2%, fluid	86.34	16.5	138.79	150.53	182.53	79.72	204.15	44.45	81.7	50.55	90.23
3	chocolate milk, fluid, low-fat milk	18.91	2.97	16.29	49.76	60.99	18.97	48.73	10.28	12.88	10.43	12.46
4	skim milk, fluid	34.39	0.3	15.35	24.68	35.63	25.76	45.46	35.29	22.25	32.7	42.98
6	yogurt, plain, low-fat	0.96	1.4	1.14	0.7	0.32	0.43	0	2.21	0.71	1.48	1.07
7	milk shake, chocolate, fast-food type	4.54	0	0.92	3.46	3.76	4.66	7.45	2.17	3.04	2	3.62
8	evaporated milk, canned	0.33	0	1.05	0.49	0.13	0	0	0.09	0	0.12	0.21
10	cheese, American, processed	5.65	0.79	4.04	4.67	5.86	5.66	8.53	6.25	9.84	3.99	6.94
11	cottage cheese, creamed, 4% milk fat	2.5	0.54	0.94	1.68	0.52	0.53	1.04	2.04	1.81	1.73	2.8
12	cheese, Cheddar, (sharp/mild)	2.35	0.5	2.74	2.6	3.3	2.03	3.44	2.56	2.88	2	3.12
13	beef, ground, regular hamburger, cooked in patty shape	9.75	0.82	4.31	8.55	9.03	10.3	20.16	7.49	13.84	7.98	12.92
14	beef chuck roast, oven roasted	7.75	0.65	2.43	2.21	2.55	7.48	6.31	4.03	11.83	8.7	12.29
16	beef (loin/sirloin) steak, pan cooked with added fat	9.82	0.05	2.7	3.96	7.03	5.5	8.78	9.15	12.43	6.86	17.21
17	pork, ham, cured, not canned, oven cooked	3.27	0.34	1.02	2.04	2.28	1.77	4.21	2.12	2.31	3.72	5.1
18	pork chop, pan cooked with added fat	2.92	0.15	1.94	2.42	1.61	2.21	2.74	1.36	3.8	1.9	3.01
19	pork sausage, (link/bulk), oven cooked	2.48	0.2	1.23	1.55	3.03	2.26	1.59	2.15	3.05	1.72	3.84
20	pork, bacon, oven cooked	1.55	0.04	0.75	0.73	1.01	0.88	2.13	1.09	2.09	1.08	2
21	pork roast, loin, oven cooked	3.7	0.14	1.4	1.3	2.32	2.77	5.12	2.84	5.22	3.67	4.94
22	lamb chop, pan cooked with added fat	0.5	0	0.08	0.1	0.59	0	1.11	0.46	1.55	0.46	0
24	chicken, drumsticks and breasts, breaded and fried, homemade	6.45	0.28	4.09	4.44	4.29	5.39	7.07	6.72	13.02	4.88	9.17
26	turkey breast, oven roasted	4.18	0.25	1.37	1.23	2.87	2.11	8.05	2.65	4.37	3.46	9.12
27	liver (beef/calf), pan fried with added fat	0.56	0.06	0.06	0.26	0.38	0.19	0	0.22	0.56	1.07	0.27
28	frankfurters, (beef/beef and pork), boiled	4.41	0.99	7.62	7.27	5.42	2.92	5.25	2.89	5.78	3.55	5.37
29	bologna	3	0.67	1.95	2.15	3.22	1.86	3.24	1.26	3.9	1.54	4.07
30	salami, lunch meat type, regular, not hard	2.65	0.06	1.26	2.17	2.2	1.66	4.84	1.86	3.3	1.61	4.46
32	tuna, canned in oil, drained	3.31	0.21	1.14	1.1	3.34	2.94	1.76	2.96	2.91	3.17	3.54
34	fish sticks, commercial, frozen, oven cooked	1.9	0.26	1.37	2.09	0.42	2.41	1.41	1.24	1.92	1.19	0.78
35	eggs, scrambled with added milk and fat	6.81	2.88	7.41	4.65	4.97	3.83	6.02	6.36	12.19	5	6.24
36	eggs, fried with added fat	6.98	2.23	6.98	5.46	7.03	5.28	6.82	5.3	7.46	5.66	11.04
37	eggs, soft boiled	1.8	0.53	1.42	1.56	0.59	1.1	0.45	1.8	2.04	1.81	1.85
38	pinto beans, boiled from dried	4.99	0.33	3.72	1.5	1.51	3.08	3.06	4.2	9.84	4.25	6.84
39	pork and beans, canned	6.93	0.64	3.54	6.29	4.11	7.39	7.03	5.58	13.08	8.44	9.25
42	lima beans, immature, frozen, boiled	0.81	0.04	0.36	0.79	0.23	0.31	0	1.4	0.82	0.63	1.09
46	peas, green, frozen, boiled	3	0.83	2.31	2.78	2.84	1.56	1.54	2.12	4.71	2.47	3.59
47	peanut butter, creamy, commercial in jar	1.98	0.19	2.87	4.04	3.09	1.67	2.91	0.9	1.81	0.74	2.03
48	peanuts, dry roasted in jar, salted	0.81	0	0.22	0.39	1.14	0.14	1.27	1.08	1.4	0.74	1.67
50	rice, white, enriched, cooked	23.24	3.58	14.93	16.09	13.56	19.05	21.73	28.44	31.25	24.49	28.42
51	oatmeal, cooked	10.33	5.12	12.53	11.65	6.31	3.92	2.67	4.33	6.88	5.1	6.93
52	farina, enriched, cooked	2.81	2.5	3.47	1.56	0.97	1.73	2.1	1.83	2.56	1.15	1.8
53	corn grits (hominy grits), enriched, cooked	3.18	3.06	4.86	2.64	5.64	1.91	2.65	1.71	2.12	2.71	3.88

U.S. Food and Drug Administration Total Diet Study Food Consumption Amounts (g/day)

TDS Diets, Version 2 (1990 food list + 1994-96, 1998 CSFII data)

OLD FOOD LIST 1990-2002

TDS food #	Food Description	Total US	6-11 mo. INCL addons	M/F 2 yr INCL addons	M/F 6 yr	M/F 10 yr	F 14-16 yr	M 14-16 yr	F 25-30 yr	M 25-30 yr	F 40-45 yr	M 40-45 yr
54	corn, (fresh/frozen), boiled	8.04	0.69	6.78	9.48	11.14	5.77	9.44	8.41	8.01	6.77	12.01
56	corn, cream style, canned	0.7	0.11	0.32	0.51	0.63	1.44	0.48	0	0.35	0.84	1.59
57	popcorn, popped in oil	2.1	0.05	1.12	1.95	2.85	1.14	2.39	2.02	2.68	2.27	2.08
58	white bread, enriched	22.06	2.7	14.74	21.34	23.29	20.1	21.07	17.08	26.71	19.36	29.05
59	rolls, white, soft, enriched	11.64	0.37	3.44	9.65	11.85	10.57	22.06	10.41	18.74	10.1	17.14
60	corn bread, southern style, homemade	3.7	0.21	1.34	2.09	0.33	4.06	4.48	1.94	2.42	2.95	5.79
61	biscuits, baking powder, enriched, refrigerated type, baked	3.31	0.15	1.04	2.05	1.81	3.13	4.74	2.76	4.73	3.66	4.39
62	whole wheat bread	7.03	0.67	4.31	5.08	4.88	3.96	6.5	4.94	9.83	5.79	6.7
63	tortilla, flour	1.98	0.08	1.14	1.67	1.56	1.63	3.94	2.26	3.72	1.83	2.8
64	rye bread	1.27	0.01	0.12	0.23	0.19	0.1	0.65	0.93	0.78	0.74	2.5
65	muffins (blueberry/plain)	4.63	0.24	3.92	6.6	7.32	5	7.74	3.63	5.15	5.22	5.96
66	saltine crackers	1.44	0.44	1.04	0.9	0.52	1.09	1.1	1.74	1.35	1.13	2.14
67	corn chips	5.56	0.56	3.26	5.05	7.89	11.72	12.11	6.29	14.08	3.9	6.01
68	pancakes made from mix with addition of egg, milk, and oil	6.34	1.13	6.88	9.85	13.81	6.43	11.73	3.64	4.15	4.13	9.02
69	noodles, egg, enriched, cooked	4.77	0.78	3.41	2.95	4.89	2.95	3.16	6.68	3.59	4.73	5.3
70	macaroni, enriched, cooked	14.67	1.91	8.04	13.17	13.83	12.25	14.45	12.38	19.82	12.37	20.61
71	corn flakes	3	0.24	2.65	4.24	3.7	2.4	6.5	2.39	3.66	1.63	2.69
72	fruit flavored, presweetened cereal	3.05	0.22	5.45	9.14	11.8	5.81	12.6	1.85	2.72	0.57	0.7
73	Shredded Wheat cereal	3.03	0.15	1.1	1.9	2.5	2.22	2.66	3.38	2.49	2.46	3.3
74	Raisin Bran cereal	2.24	0	0.99	0.51	1.95	1.06	2.58	2.3	1.11	2.51	2.18
75	crisped rice cereal	0.91	0.06	0.86	2.18	2.77	1.03	1.31	0.69	0.42	0.76	0.66
76	granola, with raisins	1.71	0.02	0.52	1.39	1.2	1.72	2.87	1.68	1.91	1.21	2.05
77	oat ring, unsweetened cereal	2.33	1.45	4.27	3.42	4.31	2.22	4.37	1.97	1.6	1.76	3.38
78	apple, red with peel, raw	13.91	1.37	18.55	19.78	15.46	10.56	10.1	9.44	14.78	14.54	12.2
79	orange, raw, (navel/Valencia)	7.01	1.13	7.2	6.55	5.47	4.84	6.67	4.69	9.55	6.96	8.83
80	banana, raw	16.05	8.32	20.57	12.3	14.36	6.46	7.08	12.56	11.11	18.53	18.56
81	watermelon, raw	7.33	0.9	4.44	7.99	3.67	1.92	12.74	6.24	1.89	8.8	7.83
83	peach, raw	3.1	0.77	1.54	1.67	0.83	3.22	1.73	3.76	1.63	4.63	3.6
84	applesauce, bottled	3.72	6.47	5.42	6.11	5.55	1.1	2.56	1.72	1.17	1.12	1.73
85	pear, raw	2.22	0.37	2.16	0.83	4.38	0.41	0.61	1.38	1.16	1.2	1.9
86	strawberries, raw	2.8	0.29	2.06	1.65	2.59	2.51	1.97	2.88	0.95	3.79	1.95
87	fruit cocktail, canned in heavy syrup	3.03	0.71	3.57	4.16	5.75	3.37	2.32	2.25	0.57	1.4	3.21
88	grapes, (purple/green), raw	3.55	0.39	6.23	3.81	4.26	3.51	0.78	2.74	1.15	4.05	3.23
89	cantaloupe, raw	5.57	0.54	2.99	4.91	1.9	5.01	4.28	3.93	2.68	8.58	7.27
91	plums, purple, raw	0.62	0.09	0.37	1.06	0.16	0.85	0.51	0.13	0.12	0.57	0.89
92	grapefruit, raw	3.11	0.14	0.31	0.28	0.29	0.4	2.01	1.95	1.7	3.63	3.07
93	pineapple, canned in juice pack	2.45	0.27	2.18	1.22	3.67	0.94	0.55	2.23	0.85	2.98	2.82
94	cherries, sweet, raw	0.26	0.03	0.19	0.32	0.19	0.18	0.03	0.02	0.12	0.28	0.52
95	raisins, dried	0.64	0.1	1.39	0.65	0.22	0.32	0.18	0.23	0.96	0.46	0.55
96	prunes, dried, uncooked	0.2	0	0.02	0.15	0	0	0	0.05	0	0.03	0

U.S. Food and Drug Administration Total Diet Study Food Consumption Amounts (g/day)

TDS Diets, Version 2 (1990 food list + 1994-96, 1998 CSFII data)

OLD FOOD LIST 1990-2002

TDS food #	Food Description	Total US	6-11 mo. INCL addons	M/F 2 yr INCL addons	M/F 6 yr	M/F 10 yr	F 14-16 yr	M 14-16 yr	F 25-30 yr	M 25-30 yr	F 40-45 yr	M 40-45 yr
97	avocado, raw	0.59	0.04	0.22	0.16	0.13	1.12	0.69	0.91	1.15	1.04	0.61
98	orange juice, frozen, reconstituted	54.62	5.32	54.8	52.03	65.21	75.79	86.92	49.6	78.76	41.98	46.05
99	apple juice, bottled	20.89	28.56	96.37	46.09	20.67	28.32	21.45	18.68	11.05	7.12	21.1
100	grapefruit juice, frozen, reconstituted	2.59	0.13	0.43	0.48	0	0	1.19	2.78	0.7	2.7	2.98
103	prune juice, bottled	0.46	0.12	0.19	0	0	0	0	0	0	1.14	0
105	lemonade, frozen, reconstituted	9.55	0.06	4.31	6.52	4.29	18.19	11.23	6.26	6.63	11.03	8.83
107	spinach, (fresh/frozen), boiled	1.94	0.33	0.46	0.63	0.37	0.36	2.02	1.7	2.36	1.65	1.75
108	collards, (fresh/frozen), boiled	1.47	0.1	0.87	0.51	0.22	2.1	0.08	0.33	0.96	1.67	0.93
109	lettuce, raw	14.82	0.01	2.22	3.9	7.35	10.39	9.57	13.4	18.41	22.92	22.19
110	cabbage, boiled from raw	2.08	0.11	0.58	0.72	0.47	0.3	0.26	0.76	1.55	2.25	1.24
111	coleslaw with dressing, homemade	3.38	0	0.65	0.45	1	1.1	2.3	2.2	4.93	3.19	5.65
112	sauerkraut, canned	0.41	0	0.13	0	0.04	0	0	0.14	0.29	0.22	0.62
113	broccoli, (fresh/frozen), boiled	5.64	0.74	3.64	3.5	4.37	1.98	3.33	7.68	4.25	6.74	5.45
114	celery, raw	0.93	0	0.33	0.42	0.74	0.16	0.8	1.09	1.05	0.89	0.89
115	asparagus, (fresh/frozen), boiled	0.76	0.11	0.23	0.13	0.21	0.49	0.36	0.35	0.75	0.86	1.03
116	cauliflower, (fresh/frozen), boiled	1.08	0.1	0.28	0.48	0.12	0.51	0.14	0.83	0.88	1.38	1.33
117	tomato, raw	11.49	0.37	2.45	2.21	5.91	7.55	9.76	9.27	11.8	13.82	15.8
119	tomato sauce, plain, bottled	8.48	1.03	4.48	5.74	7.04	6.06	7.77	7.95	10.74	7.71	13.72
121	beans, snap green, (fresh/frozen), boiled	7.16	1.57	5.74	5.78	4.11	4.32	4.15	6.23	5.81	6.99	9.28
123	cucumber, raw, pared	3.15	0.03	0.88	1.55	1.62	1.41	2.17	1.66	4.2	3.53	3.89
124	squash, summer, (fresh/frozen), boiled	2.39	0.93	0.29	0.56	0.13	0.68	1	2.34	1.62	2.46	2.24
125	sweet pepper, green, raw	1.99	0.01	0.19	0.26	0.73	0.36	1.18	1.82	3.79	3.93	2.91
126	squash, winter, (Hubbard/arorn), (raw/frozen), boiled	0.63	0.06	0.1	0.04	0	0	0.39	0.39	0.5	0.55	0.45
128	onion, raw	3.42	0.07	0.39	0.53	0.8	2.32	3.04	4.03	5.92	3.88	4.82
132	radish, raw	0.16	0	0.01	0.03	0.06	0.03	0.17	0.06	0.07	0.21	0.25
134	French fries, frozen, commercial, heated	9.89	0.77	6.61	9.04	8.09	12.03	15.61	11.12	12.83	6.31	15.14
135	mashed potatoes with margarine and milk, prepared from instant	14.74	6.22	9.44	11.4	12.7	10.04	19.86	9.51	15.56	10.43	19.53
136	boiled potato without peel	7.06	2.47	3.24	2.58	4.43	4.67	3.18	5.35	6.94	7.38	7.9
137	baked potato with peel	5.02	0.31	0.98	1.43	3.23	4.53	7.46	4.55	7.59	5.15	7.36
138	potato chips, commercial	3.98	0.05	3.19	4.66	4.82	3.87	6.89	3.97	8.63	3.29	5.31
139	scalloped potatoes, homemade	7.54	0.86	1.77	1.85	2.79	4.97	14.11	5.83	7.19	7.35	9.39
140	sweet potato, baked in skin	2.55	0.68	1.03	1.07	1.34	1.6	1.52	4.04	0.83	4.27	3.05
142	spaghetti with meat sauce, homemade	14.95	6.42	22.02	18.29	14.3	20.45	22.21	10.98	25.73	7.86	19.95
143	beef and vegetable stew, homemade	9.38	1.05	4.32	5.71	3.48	4.03	11.78	14.09	16.93	9.47	13.98
146	macaroni and cheese, prepared from box mix	13.86	5.93	20.13	20.27	14.01	15.84	17.08	10.12	18.89	12.97	9.93
147	quarter-pound hamburger sandwich on white roll with garnish, fast-food type	8.1	0.09	2.68	6.35	10.03	5.81	11.12	5.21	16.49	7.6	14.14
148	meatloaf, beef, homemade	3.88	0.81	1.9	1.9	1.48	6.48	4.2	2.35	3.59	3.07	8.3
149	spaghetti in tomato sauce, canned	6.6	1.4	7.55	7.96	8.64	5.28	15.54	5.54	3.68	4.01	5.56
151	lasagna with meat, homemade	6.2	0.02	2.86	3.02	4.07	5.13	6.21	4.95	9.68	6.58	7.19

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152	chicken potpie, frozen, heated	12.56	2.58	4.52	5.55	7.15	11.64	10.39	13.88	15.13	14.73	23.29
155	chicken noodle soup, canned, condensed, prepared with water	22.41	8.62	23.43	18.08	22.86	15.54	27.51	30.96	31.86	16.73	18.49
156	tomato soup, canned, condensed, prepared with water	2.82	0.67	3.19	4.62	1.25	1.1	2.8	8.77	3.32	1.85	3.48
157	vegetable beef soup, canned, condensed, prepared with water	15.17	4.84	11.07	9.85	7.5	16.35	12.2	14.41	10.84	18.61	20.97
160	white sauce homemade	0.78	0.02	0.22	0.76	0.38	1.26	1.04	0.93	1.6	1.1	1.19
161	dill cucumber pickles	1.8	0.04	0.62	0.82	2.91	1.42	1.88	1.37	1.71	1.23	3.19
162	margarine, stick, regular (salted)	2.39	0.24	1.2	1.68	2.13	1.64	2.88	1.65	1.99	2.45	3.02
164	butter, regular (salted)	1.38	0.12	0.57	1.02	0.73	0.49	1.31	0.92	1.5	1.18	1.72
166	mayonnaise, regular, bottled	1.69	0.02	0.38	0.76	0.74	1.17	2.84	1.32	2.24	1.54	2.62
167	half & half cream	1.76	0.01	0.11	0.17	0.32	0.17	0.43	1.77	1.68	2.66	2.19
168	cream substitute, frozen	1.22	0	0.03	0.08	0.25	0.18	0.34	1.15	1.04	1.71	1.85
169	white sugar, granulated	3.2	0.16	0.5	0.71	1.32	0.99	1.65	3.41	3.74	4.1	4.76
170	pancake syrup	3.92	0.12	3.53	5.63	8.15	4.95	8.62	2.53	2.77	2.71	4.15
172	honey	0.39	0.02	0.22	0.18	0.04	0.14	0.3	0.35	0.36	0.38	0.23
173	tomato catsup	3.91	0.04	3.04	4.84	3.76	5.95	7.46	3.74	6.1	2.44	4.29
175	chocolate pudding, from instant mix	3.8	2.13	3.35	7.03	7.3	5.02	2.92	1.57	3.31	3.04	3.8
177	vanilla flavored light ice cream	6.58	0.2	2.53	4.3	7.93	6.88	5.25	5.42	3.23	8.68	7.31
178	chocolate cake with chocolate icing, commercial	2.11	0.14	0.8	2.32	3.77	1.35	2.48	3.54	2.27	2.09	2.1
179	yellow cake with white icing, prepared from cake and icing mixes	5.29	0.67	1.92	3.48	3.56	5.6	4.6	4.09	2.95	6.79	5.78
182	sweet roll/Danish, commercial	3.17	0.23	1.12	2.44	2.54	4.19	7.38	2.91	3.16	4.26	4.39
183	chocolate chip cookies, commercial	2.58	0.24	2.4	3.64	4.1	3.15	4.73	2.29	3.08	1.95	3.14
184	sandwich cookies with cream filling, commercial	2.04	0.18	2.06	2.92	3.3	2.39	3.7	2.63	1.87	1.5	2.53
185	apple pie fresh/frozen, commercial	5.11	0.07	0.38	2.41	0.59	1.75	3.78	3.71	2.16	6.33	8.43
186	pumpkin pie, fresh/frozen commercial	3.6	0.02	0.82	1.04	1.78	1.73	4.47	2.55	3.65	3.45	4.07
187	milk chocolate candy bar, plain	3.79	0.02	1.9	3.14	3.64	4.5	5.82	4.43	3.57	4.15	6.38
188	candy, caramels	0.74	0.01	0.4	0.62	1.61	0.65	1.38	0.69	0.92	0.76	0.79
190	gelatin dessert, any flavor	3.47	1.56	2.8	4.91	4.73	4.95	3.24	2.43	0.88	3.3	1.95
191	cola carbonated beverage	172.49	0.86	33.09	68.51	124.5	267.63	359.77	217.75	373.51	126.29	227.13
193	fruit drink from powder (e.g., Kool-Aid)	31.8	4.95	42.94	56.57	41.2	49.02	84.38	26.38	37.72	17.83	18.94
194	low-calorie cola carbonated beverage	68.62	0.06	3.67	9.03	23.08	33.36	25.78	116.81	80.09	142.05	106.03
196	coffee, decaffeinated, from instant	29.04	1.84	1.03	0.4	0.44	0.71	1.67	10.35	6.15	50	29.64
197	tea, from tea bag	128.36	2.4	21.73	27.63	34.19	81.22	92.27	121.5	141.52	199.69	209.01
198	beer	76.84	0	0.12	0	0	1.57	6.5	49.01	265.06	23.41	180.75
199	dry table wine	8.95	0	0	0	0	0	0	11.83	6.35	15.42	13.24
200	whiskey	2.49	0	0	0.01	0	0	0.34	1.82	6.9	1.41	6.51
202	milk-based infant formula, high iron, ready-to-feed	7.46	434.01	6.44	0	0	0	0	0	0	0	0
203	milk-based infant formula, low iron, ready-to-feed	0.71	51.22	0	0	0	0	0	0	0	0	0
205	beef, strained/junior, with/without broth or gravy	0.02	1.27	0.01	0.03	0	0	0	0	0	0	0

U.S. Food and Drug Administration Total Diet Study Food Consumption Amounts (g/day)

TDS Diets, Version 2 (1990 food list + 1994-96, 1998 CSFII data)

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207	chicken/turkey, strained/junior, with/without broth or gravy	0.03	2.96	0	0	0	0	0	0	0	0	0
211	vegetables & beef, strained/junior	0.06	4.18	0.11	0.08	0	0	0	0	0	0	0
212	vegetables and chicken, strained/junior	0.06	4.71	0.04	0	0	0	0	0	0	0	0
213	vegetables and ham, strained/junior	0.03	2.69	0.24	0	0	0	0	0	0	0	0
214	chicken noodle dinner, strained/junior	0.11	9.07	0.01	0	0	0	0	0	0	0	0
215	macaroni, tomatoes, and beef, strained/junior	0.12	9.35	0.24	0	0	0	0	0	0	0	0
216	turkey and rice, strained/junior	0.12	8.7	0.14	0	0	0	0	0	0	0	0
218	carrots, strained/junior	0.12	7.38	0.04	0	0	0	0	0	0	0	0
219	green beans, strained/junior	0.07	6.38	0	0	0	0	0	0	0	0	0
220	mixed vegetables, strained/junior	0.07	7.32	0	0	0	0	0	0	0	0	0
221	sweet potatoes, strained/junior	0.1	8.62	0.04	0	0	0	0	0	0	0	0
222	creamed corn, strained/junior	0.01	1.44	0	0	0	0	0	0	0	0	0
223	peas, strained/junior	0.05	4.23	0	0	0	0	0	0	0	0	0
224	creamed spinach, strained/junior	0.02	1.85	0	0	0	0	0	0	0	0	0
225	applesauce, strained/junior	0.17	13.04	0.15	0	0	0	0	0	0	0	0
226	peaches, strained/junior	0.13	9.78	0	0	0	0	0	0	0	0.14	0
227	pears, strained/junior	0.11	7.75	0.12	0	0	0	0	0	0	0	0
230	apple juice, strained	0.3	17.36	1.25	0	0	0	0	0	0	0	0
231	orange juice, strained	0.02	1.2	0	0	0	0	0	0	0	0	0
232	custard pudding, strained/junior	0.02	1.67	0.02	0	0	0	0	0	0	0	0
233	fruit dessert/pudding, strained/junior	0.09	7.18	0.3	0	0	0	0	0	0	0	0
235	fruit-flavored yogurt, lowfat (fruit mixed in)	6.41	6.27	9.17	8.84	8.01	2.47	2.84	7.77	5.49	7.79	5.47
236	Swiss cheese	2.03	0.06	0.99	1.11	0.68	0.91	1.44	2.4	2.7	1.99	3.27
237	cream cheese	1.99	0.08	1.06	1.51	2.12	1.71	3.06	2.15	1.7	2.06	1.77
238	veal cutlet, pan-cooked	0.4	0	0.05	0.04	0	0.32	0	0.36	0.66	0.33	0
239	ham luncheon meat, sliced	9.1	1.02	4.82	7.31	9.31	8.55	12.85	7.34	15.45	6.34	14.73
240	chicken breast, roasted	14.44	1.34	6.99	6.95	9.13	11.17	10.25	14.35	20.92	17.07	21.32
241	chicken nuggets, fast-food	3.43	0.54	5.73	8.62	7.06	5.4	5.61	3.08	3.57	2.25	1.82
242	chicken, fried (breast, leg, and thigh), fast-food	2.04	0.01	1.08	0.47	0.84	2.84	3.3	1.56	3.3	2.3	3.05
244	shrimp, boiled	3.34	0.04	0.49	0.87	1.09	1.81	2.3	7.85	4.86	3.79	3.63
245	kidney beans, dry, boiled	1.82	0.2	0.82	0.87	0.98	3.56	1.58	1.34	1.08	1.88	3.33
246	peas, mature, dry, boiled	1.46	0.39	0.51	0.84	0.76	0.64	1.3	0.34	1.88	1.14	1.72
247	mixed nuts, no peanuts, dry roasted	0.81	0	0.3	0.38	0.39	0.35	0.19	0.91	1.2	0.67	1.34
248	cracked wheat bread	2.82	0.18	0.95	1.83	1.33	1.36	1.81	1.95	2.43	2.67	3.84
249	bagel, plain	3.42	0.31	0.87	1.94	1.86	2.68	2.1	3.18	3.73	5.42	4.11
250	English muffin, plain, toasted	1.25	0	0.27	0.52	0.55	0.79	3.13	0.79	0.73	1.32	1.26
251	graham crackers	0.76	1.35	1.8	1.81	0.63	0.79	0.88	0.38	0.49	0.94	0.44
252	butter-type crackers (e.g., Ritz, Hi-Ho)	2.16	0.79	2.86	2.65	1.95	2.14	2.36	2.69	1.89	2.1	2.15
253	apricot, raw	0.21	0.11	0.06	0.22	0	0	0	0.05	0.12	0.13	0.05
254	peach, canned in light/medium syrup	2.25	0.57	3.5	4.57	4.26	1.48	0.78	0.42	1.3	1.28	2.4
255	pear, canned in light syrup	0.88	0.65	1.79	1.96	1.8	0.4	0.83	0.3	0	0.27	1.98

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256	pineapple juice, from frozen concentrate	2.5	0.83	4.39	2.12	2.33	2.93	2.24	3.18	3.47	1.46	2.98
257	grape juice, from frozen concentrate	11.95	3.49	26.06	8.33	8.54	11.74	8	19.79	14.82	9.66	7.83
258	French fries, fast food	9.15	0.96	7.03	8.69	11.04	12.48	18.31	9.23	22.89	6.02	8.36
259	carrot, fresh, boiled	5.17	1.94	3.43	5.24	5.22	2.51	3.15	5.22	4.31	5.72	6.53
260	tomato, stewed, canned	3.74	0.08	0.38	0.71	1.79	3.94	2.49	5.35	6.96	4.07	4.98
261	tomato juice, bottled	2.3	0.04	0.07	0.2	0	0.02	0.3	4.13	4.62	1.37	2.98
262	beets, fresh/frozen, boiled	0.51	0	0.06	0.02	0.03	0.09	0.12	0.28	0.14	0.68	0.52
263	Brussels sprouts, fresh/frozen, boiled	0.22	0.06	0.01	0	0	0.52	0.91	0	0	0.48	0.34
264	mushrooms, raw	0.75	0.01	0.09	0.07	0.03	0.14	0.31	1.55	0.87	1.03	0.69
265	eggplant, fresh, boiled	0.85	0.01	0.18	0	0.04	0.22	0	0.03	1.45	0.95	1.3
266	turnip, fresh/frozen, boiled	0.15	0	0.08	0.04	0	0	0	0.11	0	0.28	0.06
267	okra, fresh/frozen, boiled	0.42	0.07	0.14	0.16	0.03	0.17	0.61	0.25	0.33	0.71	0.16
268	mixed vegetables, frozen, boiled	4.48	0.97	1.63	1.75	2.39	2.3	2.35	4.95	4.16	5.65	3.78
269	beef stroganoff, homemade	3.28	0.27	1.41	2.98	1.04	1.79	2.55	2.66	5.73	3.67	5.15
270	green peppers stuffed with beef and rice, homemade	2.31	0	0.46	1.91	0.83	2.75	0.94	0.8	2.71	3.76	1.02
271	chili con carne with beans, homemade	7.27	0.26	1.47	5.06	6.66	5.94	10.92	5.09	8.35	3.71	14.33
272	tuna noodle casserole	3.68	0	1.44	1.28	2.25	4.83	7.36	1.84	4.46	2.35	5.56
273	frozen meal-salisbury steak with gravy, potatoes, and vegetables, heated	0.98	0	0	0.12	0	0.95	0	0.74	0.4	1.66	1.37
274	frozen meal-turkey with gravy, dressing, potatoes, and vegetable, heated	1.78	0	0.19	0.31	1.63	1.6	0.44	1.23	0.88	2.3	1.68
275	quarter-pound cheeseburger on bun, fast-food	9.86	0.41	2.82	4.87	7.48	11.05	18.42	8.39	28.44	4.76	10.72
276	fish sandwich on bun, fast-food	3.36	0.05	0.48	0.9	1.12	1.23	4.13	4.08	6.47	4.75	3.82
277	frankfurter on bun, fast-food	1.45	0.04	1.96	2.35	2.88	1.62	2.17	2.14	1.05	1.89	0.41
278	egg, cheese, and ham on English muffin, fast-food	2.69	0.13	0.73	1.65	2.47	2.38	2.13	3.16	5.24	2.46	4.83
279	taco/tostada, from Mexican carry-out	11.69	0.1	2.49	6.44	14.59	16.44	19.2	13.14	23.96	12.05	9.61
280	cheese pizza, regular crust, from pizza carry-out	4.95	0.21	3.28	6.43	8.62	6.89	14.98	5.82	6.45	2.65	4.25
281	cheese and pepperoni pizza, regular crust, from pizza carry-out	13.64	0.55	5.3	12.33	14.07	16.97	37.53	12.33	29.56	11.81	12.97
282	beef chow mein, from Chinese carry-out	15	1.77	7.52	7.5	11.57	9.97	15.03	20.43	28.6	15.02	22.83
283	bean with bacon/pork soup, canned, condensed, prepared with water	4.95	0.68	1.52	1.34	1.9	0.34	2.3	7.38	4.65	5.26	5.69
284	mushroom soup, canned, condensed, prepared with whole milk	3.93	0.58	0.38	0.88	0.2	1.23	1.31	3.37	1.66	4.79	4.88
285	clam chowder, New England, canned, condensed, prepared with whole milk	2.58	0	0.6	0.44	1.06	0.99	2.38	1.03	2.57	2.62	3.19
286	vanilla ice cream	14.2	0.8	8.39	17.57	21.58	11.39	19.8	8.66	11.6	10.06	21.17
287	fruit flavor sherbet	1.17	0.07	1.14	1.19	0.56	2.54	1.63	0.07	0.88	1.14	4.07
288	Popsicle, any flavor	3.68	0.49	7.24	10.66	9.17	8.65	10.6	1.67	2.17	0.86	1.71
289	chocolate snack cake with chocolate icing (e.g., Ding Dongs)	1.38	0.07	0.95	2.01	2.67	1.75	1.24	0.56	1.35	1.05	2.05

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290	cake doughnuts with icing, any flavor, from doughnut store	3.52	0.13	1.65	2.85	3.02	5.96	6.11	2.53	3.99	2.67	4.55
291	brownies, commercial	1.03	0	0.34	2.04	1.3	1.86	1.81	0.73	0.49	0.96	2.06
292	sugar cookies, commercial	2.88	0.72	3.43	4	3.74	2.66	2.97	2.66	2.98	2.87	2.47
293	suckers, any flavor	2.05	0.15	3.23	5.51	7.89	4.14	4.6	0.74	0.8	0.94	1.07
294	pretzels, hard, salted, any shape	2.25	0.14	1.62	1.96	1.99	2.79	4.55	2.1	2.1	2.52	2.73
295	chocolate syrup dessert topping	0.97	0.01	0.68	0.93	1.38	0.52	1.18	0.33	0.45	1.47	2.08
296	jelly, any flavor	2.21	0.19	1.87	2.93	2.4	1.41	4.21	1.76	1.99	1.44	2.06
297	sweet cucumber pickles	0.48	0	0.08	0.07	0.58	0.46	0.3	0.08	0.21	0.46	0.54
298	yellow mustard	0.96	0	0.16	0.43	0.87	0.91	1.65	0.88	1.31	0.64	2.36
299	black olives	0.37	0	0.18	0.24	0.07	0.32	0.55	0.4	0.69	0.49	0.58
300	sour cream	1.16	0.04	0.33	0.78	1.15	1.63	1.46	1.31	2.99	1.12	0.77
301	brown gravy	5.82	0.68	1.27	3.21	4.37	5.28	7.74	2.63	5.79	5.63	9.49
302	French salad dressing, regular	5.24	0.03	0.99	1.77	3.31	4.26	3.96	5.12	6.44	6.92	8.22
303	Italian salad dressing, low-calorie	1.59	0	0.14	0.35	0.54	1.18	0.76	1.69	1.49	2.82	3.12
304	olive/safflower oil	0.18	0.02	0.03	0.19	0.01	0.03	0.04	0.08	0.52	0.12	0.1
305	coffee, from ground	221.62	0	0.22	0.53	6.17	8.39	16.62	194.56	230.76	338.43	461.38
306	fruit-flavored carbonated beverage	81.61	1.57	33.02	53.5	96.41	110.55	233.64	109.91	139.18	59.62	71.51
307	fruit drink, canned (e.g., Hi-C)	40.99	20.29	54.23	77.86	81.94	79.57	95.41	27.71	49.45	24.21	28.75
308	martini	2.6	0	0	0	0	0.03	0	5.69	2.7	4.41	2.24
309	soy-based infant formula, ready-to-feed	2.27	128.08	1.25	0.19	0	0	0	0	0	0	0
311	rice infant cereal, instant, prepared with whole milk	0.14	10.88	0.14	0	0	0	0	0	0	0	0
312	rice cereal											
313	bananas with tapioca, strained/junior	0.12	9.96	0.04	0	0	0	0	0	0	0	0
314	beets, baby food	0	0.11	0	0	0	0	0	0	0	0	0
317	teething biscuits	0.01	0.55	0.06	0	0	0	0	0	0	0	0
318	salmon, fresh/frozen, steaks/fillets, baked	6.08	0.26	1.55	2.27	2.41	3.95	4.43	6.07	4.19	6.19	7.32
319	rice/apple cereal, strained/junior	0.08	7.39	0	0	0	0	0	0	0	0	0
320	squash, strained/junior	0.07	6.12	0	0	0	0	0	0	0	0	0
700	BF, cereal, barley, dry, prep w/ water	0.01	0.32	0.01	0	0	0	0	0	0	0	0
701	BF, cereal, mixed, dry, prep w/ water	0.04	3.78	0	0	0	0	0	0	0	0	0
703	BF, juice, apple-banana	0.06	2.79	0.95	0	0	0	0	0	0	0	0
704	BF, juice, apple-cherry	0.06	4.19	0.09	0	0	0	0	0	0	0	0
705	BF, juice, apple-grape	0.03	2.59	0.17	0	0	0	0	0	0	0	0
710	BF, juice, mixed fruit	0.1	6.21	0.52	0	0	0	0	0	0	0	0
711	BF, juice, pear	0.07	3.05	0.38	0	0	0	0	0	0	0	0
712	BF, juice, grape	0.06	4.05	0	0	0	0	0	0	0	0	0
713	BF, pears and pineapple	0.01	1	0	0	0	0	0	0	0	0	0
714	BF, plums w/ apples and/or pears	0.04	3.96	0.01	0	0	0	0	0	0	0	0
715	BF, bananas and pineapple	0.02	2.3	0	0	0	0	0	0	0	0	0
716	BF, apples/applesauce w/ apricots	0.07	7.29	0	0	0	0	0	0	0	0	0
717	BF, apricots w/ mixed fruit	0.03	3.37	0	0	0	0	0	0	0	0	0

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719	BF, banana dessert	0.01	0.99	0	0	0	0	0	0	0	0	0
720	BF, peach cobbler/dessert	0.01	0.73	0	0	0	0	0	0	0	0	0
721	BF, fruit yogurt dessert	0.02	1.47	0	0	0	0	0	0	0	0	0
722	BF, dutch apple/apple cobbler	0.02	1.86	0	0	0	0	0	0	0	0	0
723	BF, arrowroot cookies	0.01	0.6	0	0	0	0	0	0	0	0	0
724	BF, zweiback toast	0.01	0.09	0	0	0	0	0	0	0	0	0
	TOTAL CONSUMPTION	**2078**	**1158**	**1315**	**1483**	**1672**	**1698**	**2468**	**1923**	**2711**	**2034**	**2757**

Appendix N

PBPK Modeling of Ethylbenzene Exposure of Infants Via Breastmilk

FINAL

15 February 2006

Prepared by

Lisa M. Sweeney, Ph.D., DABT and Michael L. Gargas, Ph.D.

The Sapphire Group
Dayton, Ohio

For

The Ethylbenzene Panel
American Chemistry Council
Arlington, VA

Appendix N. PBPK Modeling of Ethylbenzene Exposure of Infants Via Breastmilk

INTRODUCTION

The transfer of ethylbenzene from maternal tissue to infants via ingestion of breastmilk (Section 6) was determined using PBPK modeling. Details of the model that are specific to this scenario are described in this Appendix.

METHODS

Model Description

The PBPK model for nursing mothers was developed by modifying the existing PBPK model of Haddad *et al.* (2000). The Haddad *et al.* model was developed based on blood concentrations in volunteers exposed to EB by inhalation. The choice of this model and its predictive performance are described in greater detail in Appendix P. To ensure that the model application was consistent with EPA default exposure guidelines, the default maternal body weight of 71.8 kg was used and total ventilation was adjusted. Alveolar ventilation was assumed to be equal to 0.6 × total ventilation.

The model for a nursing mother was constructed by adding a mammary compartment. Milk was assumed to be in equilibrium with mammary tissue and venous blood exiting the mammary tissue. Blood flow to mammary tissue was estimated as 7 percent of cardiac output, based on the model of Fisher *et al.* (1997). The blood flow to the richly perfused tissues was reduced from the value used by Haddad *et al.* (2000) to ensure mass balance. The volume of mammary tissue was taken from ICRP (1975). Milk flow rate was assumed to be constant throughout the nursing periods, at a rate sufficient to produce the average mother's milk ingestion for a 0-12 month infant specified by EPA (2002) (0.688 L/d). An average infant body weight of 8.5 kg was used (EPA, 2002) to normalize dose to body weight.

The milk:blood partition coefficient for EB was estimated as 3, based on analogy to the structurally similar compounds benzene, toluene, xylenes, and styrene, which had measured milk:blood partition coefficients of 2.04-2.98 (Fisher *et al.*, 1997). A mammary:blood partition coefficients was also required for the model. This value was derived by calculation of an estimated mammary:air partition coefficient, which was divided by the blood:air partition coefficient to derive the estimated mammary:blood partition coefficient. The mammary: air partition coefficient for EB was estimated based on the lipid content of human adipose tissue and mammary tissue (Duck, 1990). Partitioning to adipose was approximated by the partitioning to the lipid fraction (71.4 percent) alone. Mammary partitioning was likewise assumed to be primarily to the lipid fraction (30.9 percent). Thus the mammary: air partition coefficient for EB was estimated as adipose: air partition coefficient × (mammary lipid %/adipose lipid %) = 1556 × (30.9/71.4) = 673.

Activity/Location Schedules for EB Exposed Mothers

The model incorporates scheduling of nursing, eating, and moving among environments with different EB concentrations. These schedules are summarized in Table 1.

Table 1. Schedules for EB Exposed Mothers

Production Worker Schedule			**At-Home Mother Schedule**		
Time	**Activity**	**location**	**Time**	**Activity**	**location**
07:45	nurse	home	07:45	nurse	home
08:00		vehicle	08:00	meal 1	home
08:45		production	11:45	nurse	home
11:45	nurse	home/indoor air	12:00	meal 2	home
12:00		home/indoor air	12:30		vehicle
12:45		production	14:00		outdoors
15:15		vehicle	15:30	nurse	home
16:00	nurse	home	15:45		home
16:15		outdoors	18:00	meal 3	home
17:45		home	21:45	nurse	home
21:45	nurse	home	22:00		home
22:00		home			

Breast milk ingestion by the infant was assumed to occur during four episodes per day, with durations of 15 minutes per episode, described in the model as a pulse function.

The average daily amount of time spent in different environments was specified for two groups of nursing mothers, at-home adults and production workers. The exact schedules for nursing and daily activities that were used in the modeling were developed by the PBPK modeling/risk assessment contractor. These schedules incorporate the total specified time for different environments and practical considerations. For example, motor vehicle occupancy (driving) occurs immediately before and after work. Nursing in the production area or in a motor vehicle is not likely to occur, so nursing was not scheduled in these locations.

Dietary ingestion by the mother was neglected for the production worker because of the small contribution it makes to the mother's exposure. Dietary ingestion for the at-home mother was described as occurring in three meals, consisting of 20, 40, and 40 percent of the total intake. Each meal was assumed to last 30 minutes, and the ingestion was described as a pulse function. Uptake from the stomach to the liver blood supply was modeled as first order, occurring at a rate equal to that determined for rats dosed with EB in corn oil (Faber *et al.,* 2006).

The inhalation exposure, ingestion, and nursing schedules were incorporated in the model using pulse functions. Central tendency and upper-bound estimates for dietary intake and EB concentrations in the different environments were taken from Chapters 6 and 7. The non-occupational exposure concentrations were those derived for urban smokers (those

with the highest exposures outside the workplace). Infant doses from breastfeeding were based on model simulations in which mothers had been exposed to EB under the specified daily schedule for over two weeks, in order to establish equilibrium.

RESULTS

A sample time course of mammary venous blood concentration for a mother with occupational exposure to EB is provided in Figure N-1. Breastmilk is assumed to be in equilibrium with the mammary venous blood. Note that the time starts at 336 hrs (two weeks), for establishment of equilibrium.

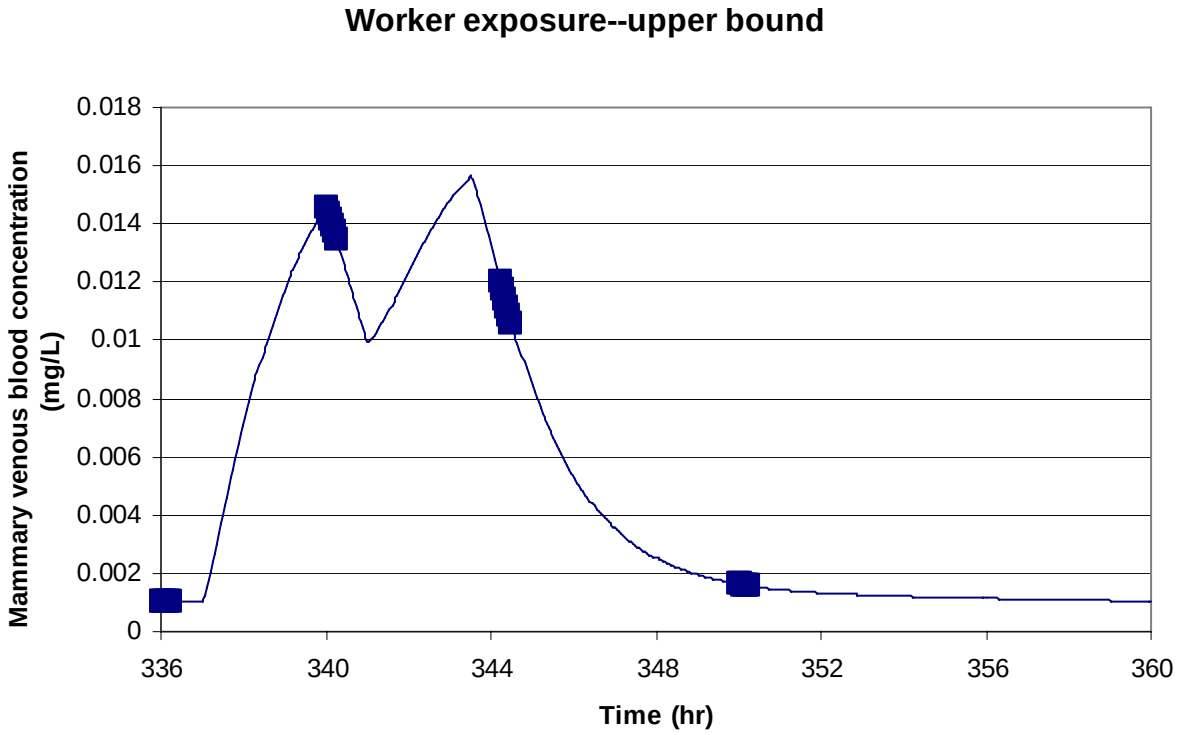

Figure N-1. Predicted mammary venous blood concentrations for worker exposure to "upperbound" concentrations of ethylbenzene. Filled squares are used to highlight blood concentrations during nursing periods.

Daily EB ingestion from breastmilk was calculated for breastfed infants with mothers who are urban smokers using the PBPK model. Results are summarized in Table N-2.

Table N-2. Infant Exposure to EB via Breastmilk Ingestion—PBPK Model Calculation

	Daily Ingested EB Dose (mg/kg/d)	
	Child of At-Home Mother	Child of Production Worker
Central Tendency	9.1×10^{-6}	1.8×10^{-4}
Upper Bound	2.0×10^{-5}	1.7×10^{-3}

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Appendix O

Robust Summaries for Selected Studies

ETHYLBENZENE TOXICITY HAZARD KEY STUDIES

[RS - 1]

Acute Oral Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	Experimental (Non-regulatory)
Type:	LD_{50}
GLP:	Pre-GLP
Year:	1962
Species/Strain:	Rat / Carworth-Wistar
Sex:	Male
#/dose:	5
Vehicle:	Unknown (water, corn oil, or a solution of 25% sodium 3,9-diethyl-6-tridecanol sulfate “Tergitol Penetrant 7”)
Route of Administration:	Oral gavage
Doses/Concentrations:	Logarithmic series differing by a factor of 2
Dose Volume Administered:	Single dose
Post Dose Observation Period:	14 days
Results (LD50):	5.46 g/kg bwt (5.09 – 5.86) (This value is noted in some secondary sources as 4.7 g/kg bwt, presumably due to misinterpretation of the study table finding and inappropriate conversion of the value from mL/kg to g/kg. The table value for ethylbenzene is given as 5.46 (5.09 – 5. 86)* under a column header units of mL/kg; the * footnote at the end of the table, however, notes “as gm/kg in a suitable vehicle”, so conversion is not appropriate).
Remarks:	The animals were 4 – 5 weeks of age and weighed 90 -120 g and were not fasted prior to dosing. The most probable LD_{50} value and the fiducial range were estimated by the method of Thompson using the tables of Weil. The figures in parentheses show limits of ± 1.96 standard deviations.

Conclusion:	The acute oral LD_{50} for ethylbenzene in male rats is 5.46 g/kg bwt (5.09 – 5.86).
Data Quality:	2 – Reliable study with restrictions. Study is pre-GLP but sufficiently documented and meets generally accepted scientific principles.
Reference:	Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C. and Striegel, J.A. (1962). Range finding toxicity data: List VI. *Am. Ind. Hyg. Assoc. J.* 23:95-107.

[RS - 2]

Acute Dermal Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	Experimental (Non-regulatory)
Type:	LD_{50}
GLP:	Pre-GLP
Year:	1962
Species/Strain:	Rabbit / New Zealand White
Sex:	Male
#/dose:	4
Vehicle:	Unknown (water, corn oil, or a solution of 25% sodium 3,9-diethyl-6-tridecanol sulfate “Tergitol Penetrant 7”)
Route of Administration:	Skin
Doses/Concentrations:	Logarithmic series differing by a factor of 2
Dose Volume Administered:	Single dose
Post Dose Observation Period:	14 days
Results (LD50):	17.8 mL/kg bwt (Equivalent to 15.3 g/kg bwt)
Remarks:	The animals weighed 2.5 – 3.5 kg. Technique similar to the one-day cuff method of Draize and associates. The fur was removed from the entire trunk by clipping and the dose was retained beneath an impervious plastic film. The animals were immobilized during the 24-hour contact period, after which the film was removed. The most probable LD_{50} value was estimated by the method of Thompson using the tables of Weil.
Conclusion:	The acute dermal LD_{50} for ethylbenzene in male rabbits is 17.8 mL/kg bwt (15.3 g/kg bwt).
Data Quality:	2 – Reliable study with restrictions. Study is pre-GLP but sufficiently documented and meets generally accepted scientific principles.

Reference:	Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C. and Striegel, J.A. (1962). Range finding toxicity data: List VI. *Am. Ind. Hyg. Assoc. J.* 23:95-107.

[RS - 3]

Acute Inhalation Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	Experimental (Non-regulatory)
Type:	LC_{50}
GLP:	Pre-GLP
Year:	1962
Species/Strain:	Rat / Carworth-Wistar
Sex:	Males or Females
#/dose:	6
Vehicle:	Air
Route of Administration:	Inhalation
Doses/Concentrations:	In an essentially logarithmic series differing by a factor of 2
Exposure Time:	4 hours
Post Dose Observation Period:	14 days
Results (LC50):	4000 ppm
Remarks:	The animals' age and weight ranges were not provided. Inhalation was by metered vapor concentrations conducted with flowing streams of vapor prepared by proportioning pumps. Concentrations recorded were nominal and not analytically verified. Results given as fractional mortality among 6 rats within 14 days. 4000 ppm ethylbenzene administered for 4 hours yielded 14 day mortality in 3 of 6 rats.
Conclusion:	The acute inhalation LC_{50} for ethylbenzene in rats is 4000 ppm.
Data Quality:	2 – Reliable study with restrictions. Study is pre-GLP but sufficiently documented and meets generally accepted scientific principles.
Reference:	Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C. and Striegel, J.A. (1962). Range finding toxicity data: List VI. *Am. Ind. Hyg. Assoc. J.* 23:95-107.

[RS - 4]

Genetic Toxicity - *In Vitro* Gene Mutation: Bacterial Reverse Mutation Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99%
Method:	EU Annex V.B.14. OECD Guideline 471 - Genetic Toxicology: *Salmonella typhimurium* Reverse Mutation Assay
Type:	Bacterial Reverse Mutation Test
System of Testing:	Bacteria
GLP:	Yes
Year:	1992
Species/Strain:	*S. typhimurium* / TA97, TA98, TA100, TA1535, TA1537
Metabolic Activation:	With and Without
Species/cell type:	Male Sprague-Dawley Rat and Syrian Hamster / Liver; Aroclor 1254-induced (S-9 fraction)
Concentrations: **Tested** **Vehicle**	 10 to 10000 µg/plate Distilled water
Remarks for Test Conditions:	Control plates were set up with solvent alone and with an appropriate known positive control compound. The S9 fractions were prepared from Aroclor-induced rats and hamsters. Ethylbenzene was tested initially in a toxicity assay with a range of test concentrations to establish the appropriate dose range for the mutagenicity assay. Ethylbenzene was tested at half-log dose intervals up to 10000 µg/plate. The mutagenicity assay was then performed based on the results of the toxicity assay taking into account the effect on cell viability and any possible positive increases in mitotic gene conversion. Control plates were set up with solvent alone and with the positive control compounds in the absence of S-9 activation (sodium azide for TA1535 and TA100; 9-aminoacridine for TA97 and TA1537; 4-nitro-o-phenylenediamine for TA98) and with S-9 metabolic activation (2-aminoanthracene for all strains). Ethylbenzene was designated non-mutagenic only after it had been tested in strains TA98, TA100, TA1535, TA97 and TA1537 without activation, and with 10% and 30% rat and hamster S-9 activation.
Results:	Negative

Remarks:	
Conclusion:	Ethylbenzene did not induce reverse gene mutations in bacteria.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data
Reference:	Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environmental and Molecular Mutagenesis*, Volume 19 (Supplement 21):2-141.

[RS - 5]

Genetic Toxicity - *In Vitro* Gene Mutation: Bacterial Reverse Mutation Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99%
Method:	Not specified
Type:	Bacterial Reverse Mutation Test
System of Testing:	Bacteria
GLP:	Yes
Year:	1985
Species/Strain:	*Escherichia coli* WP2, WP2uvra
Metabolic Activation:	With and Without
Species/cell type:	Rat / Liver (S9 Fraction)
Concentrations: **Tested** **Vehicle**	 0.2 to 2000 µg DMSO
Remarks for Test Conditions:	The article reports on the laboratory's testing of 41 compounds over a 5 year period. The test methods were modified over time with one bacterial assay method used from 1975-1980 and variations to this method employed after 1980. The tests for ethylbenzene were conducted during 1979-1980; hence the first method is presumed to have been used and is described below. The method used was the Plate-Incorporation Assay, using S9 microsomal fraction obtained from a rat liver homogenate from rats pre-treated with Aroclor 1254. A range of amounts of test compound were tested (0.2, 2, 20, 500 and 2000 µg/plate) both in the presence and absence of S9 mix. Overnight broth cultures were washed and resuspended in phosphate buffer pH 7.0. The suspension was then distributed in 2 mL volumes into universal containers and 20 µL test compound solution was added (-S9). For the tests incorporating microsomal activation (+S9), 0.5 mL S9 mix was added to each 2 mL bacterial suspension culture together with 25 µL test compound solution. All cultures were incubated at 37ºC for 1 hour before 0.1 mL volumes were seeded onto minimal agar plates with the appropriate amino acid supplement. Appropriate dilutions were placed onto nutrient agar to determine the numbers of survivors. The plates were then incubated at 37ºC before the colonies were counted.

	In the mutation assay control plates were set up with the solvent alone and with a known positive control compound (specific compound not stated). All tests were carried out in quadruplicate. Two replicate assays were carried out on different days in order to confirm the reproducibility of results. Data were interpreted on the basis of a consistent doubling of the spontaneous reversion frequency confirmed by a dose-response relationship. A positive response was given in cases where the number of induced revertants was less than twice the spontaneous rate, but a reproducible dose-related increase in revertants was detected.
Results:	Negative
Remarks:	
Conclusion:	Ethylbenzene did not induce reverse gene mutations in bacteria.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Dean, B.J., Brooks, T.M., Hodson-Walker, G. and Huston, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. *Mutat. Res.* 153:57-77.

[RS - 6]

Genetic Toxicity - *In Vitro* Gene Mutation: Yeast Mitotic Gene Conversion

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99 %
Method:	Not specified
Type:	Yeast Mitotic Gene Conversion Assay
System of Testing:	*Saccharomyces cerevisiae*
GLP:	Yes
Year:	1985
Species/Strain:	*Saccharomyces cerevisiae*
Metabolic Activation:	With and Without
Species/cell type:	Rat / Liver (S9 Fraction)
Concentrations: **Tested** **Vehicle**	 0.01 to 5 mg/mL DMSO
Remarks for Test Conditions:	Yeast cells were grown in log phase, washed and re-suspended in 2.5 strength YEPD broth at a concentration of 10 x 10^6 cells/mL. The suspension was then divided into 1.9 mL amounts in 30 mL universal containers and 0.1 mL of the test compound solution was added (-S9). For the experiments with metabolic activation (+S9), 0.1mL of test compound was added to 1.6 mL of yeast cell suspension, together with 0.3 mL S9 mix (prepared from the livers of Aroclor-induced rats). The cultures were incubated with shaking at 30 ºC for 18 hours. Aliquots were then plated onto YM plates supplemented with either histidine or tryptophan to determine the number of prototrophs as each locus, and dilutions were spread onto YEPD plates to determine cell viability. Initially a range of concentrations of test compound (0.01, 0.1, 0.5, 1.0, and 5.0 mg/mL) were tested as solubility allowed. A second experiment was then performed based on the initial test results taking into account the effect of the chemical on cell viability and any possible positive effect. The test compound was considered to increase the rate of mitotic gene conversion if there was a reproducible dose-related increase in the number of prototrophs per 10^6 survivors together with an increase in the number of prototrophs per plate.

Results:	Negative
Remarks:	
Conclusion:	Ethylbenzene did not induce mitotic gene conversion in yeast.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Dean, B.J., Brooks, T.M., Hodson-Walker, G. and Huston, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. *Mutat. Res.* 153:57-77.

[RS - 7]

Genetic Toxicity - *In Vitro* Gene Mutation: Mammalian Cell Mutation Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	Not specified
Type:	Cell Mutation Assay at the Thymidine Kinase Locus (tk+/-)
System of Testing:	Mouse Lymphoma Cells L 5178Y
GLP:	Not specified
Year:	1988
Species/Strain:	Mouse Lymphoma Cells L 5178Y
Metabolic Activation:	Only Without
Species/cell type:	Not applicable
Concentrations: **Tested** **Vehicle**	 10 to 80 µg/mL (highest non-lethal concentration) DMSO
Remarks for Test Conditions:	An initial toxicity test was conducted to measure cell population expansion. Experimental Design: The experiment consisted of the following groups: vehicle control, four cultures; positive control, two cultures; at least 5 test compound concentrations, two cultures per concentration. Positive controls included ethyl methanesulphonate (EMS; 250 µg/mL) and methyl methansulphonate (MMS, 15 µg/mL). Trial I without S9 mix: 10, 20, 40, 80, 160 µg/mL Trial 2 without S9 mix: 20, 40, 60, 80, 100 µg/mL Mutation Experiment: Exposure: Each exposed culture consisted of 6 x 10^6 cells in a final volume of 10 mL Fischer's medium containing 5% heat-inactivated horse serum (F_{5P}) in a 30 mL screw-cap plastic tube. The tube was incubated for 4 hours on a horizontal axis roller drum rotating at 10 rpm. At the end of the incubation period, the cells were sedimented by centrifugation at 500 g.av. for 10 minutes, washed, and finally resuspended in 10 mL Fischer's medium containing 10% heat-

	inactivated horse serum (F_{10P}). The cell suspensions (3 x 10^5 cells/mL) were incubated for a 2 day expression period, the cell population density being adjusted back to 20 mL of 3 x 10^5 cells/mL after 24 hours. After 48 hours, the cell population densities were estimated and culture volumes containing 3 x 10^6 cells adjusted to 15 mL with F_{10P}, giving a cell population density of 2 x 10^5. Cloning Efficiency: A 0.1 mL sample of the cell suspension was withdrawn and diluted 1:100. Three 0.1 mL samples (200 cells) of the diluted cultures were transferred to 30 mL tubes, mixed with 25 mL cloning medium containing 10% heat inactivated horse serum containing 0.35% Noble agar and poured into 90 mm Petri plates. Mutant Selection: Three aliquots (each containing 10^6 cells) of the remaining culture were distributed to 30 mL tubes, mixed with 10 mL cloning medium to give final concentrations of 0.35% Noble agar and 3 ug trifluorothymidine/mL, then poured into 90 mm Petri plates. Incubation: The agar was gelled at 4ºC for 5-10 minutes, and then the plates were incubated for 11-14 days in 5% CO_2:95% air at 37ºC. Colony Counting: Colonies were counted using an automated colony counter. Calculations: The Relative Total Growth and Mutant Fraction were calculated. A test was considered positive when, out of 3 trials, a positive trial was reproducible. Statistics: Statistical analyses consisted of a dose-trend test and a variance analysis of pair-wise comparisons of each dose against the vehicle control.
Results:	Positive
Remarks:	Ethylbenzene was mutagenic in two experiments without S9 mix only at the highest non-lethal concentration (80 ug/mL). At this concentration there was significant cytotoxicity, with the relative total growth in two trails being 34 or 13% of the control level. Average Mutant Fraction Trial 1 / Trial 2 DMSO: 60 / 31 10 µg/mL: 56 20 µg/mL: 53 / 43 40 µg/mL: 67 / 29 60 µg/mL: / 43 80 µg/mL: 589* / 150* 100 µg/mL: / lethal 160 µg/mL: lethal EMS: 149* / 225* MMS: 145* / 107* * Statistically significantly higher ($p < 0.05$) than control
Conclusion:	Ethylbenzene induced mutations in mouse lymphoma cells only at the highest non-lethal and cytotoxic concentration.

Data Quality:	2 – Reliable with restrictions. Protocol not current on appropriate toxicity levels and evaluation criteria. Positive results accompanied by substantial increases in cytotoxicity that complicate interpretation.
Reference:	McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. and Caspary, W.J. (1988). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay. III: 72 coded chemicals. *Environ. Mol. Mutag.* 12:85-154.

[RS - 8]

Genetic Toxicity - *In Vitro* Gene Mutation: Mammalian Cell Mutation Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7%
Method:	OECD Guideline 476 – *In Vitro* Mammalian Cell Gene Mutation Test
Type:	Cell Mutation Assay at the Thymidine Kinase Locus (tk+/-)
System of Testing:	Mouse Lymphoma Cells L 5178Y (soft agar method)
GLP:	Yes
Year:	2000
Species/Strain:	Mouse Lymphoma Cells L 5178Y
Metabolic Activation:	With and Without
Species/cell type:	Male Wistar Rat / Liver; Phenobarbital and β-Naphthoflavone-induced (S-9 fraction)
Concentrations: **Tested** **Vehicle**	 8.6 to 100 µg/mL (without S9 mix) 68.8 to 900 µg/mL (with S9 mix) DMSO
Remarks for Test Conditions:	The study investigated the potential of ethylbenzene to induce mutations at the thymidine kinase (tk) locus in L5178Y tk+/- mouse lymphoma cells using six independent experiments, a treatment period of 4 hours, an expression and growth period of 72 hours and two parallel cultures. According to an initial range finding cytotoxicity test for the determination of the experimental doses and taking into account the cytotoxicity actually found in the first main experiment, the following doses were evaluated: <u>Experiment I</u> without S9 mix: 8.6, 17.2, 34.4, 68.8 µg/mL with S9 mix: 68.8, 137.5, 275, 550, 825 µg/mL <u>Experiment II</u> without S9 mix: 45, 60, 75, 90, 100 µg/mL with S9 mix: 300, 450, 600, 750, 900 µg/mL The third experiment was carried out selecting the same dose

	ranges as in the second experiment to clarify the results of the first two experiments. However, due to excessively strong toxic effects with S9 mix, this experiment part (with S9 mix) had to be terminated prior to the generation of experimental data. Experiment III without S9 mix: 45, 60, 75, 90, 100 µg/mL Experiments IV and V which were performed solely in the presence of metabolic activation also had to be terminated right after treatment since very few surviving cells were detectable. Therefore experiment VI was carried out with a dose range adjusted to the toxic effects observed in experiments III to V. Experiment VI with S9 mix: 25, 50, 75, 100, 150 µg/mL Concurrent negative and solvent controls (DMSO) were performed. Positive control substances were methylmethane sulfonate (without metabolic activation) and 3-methylcholanthrene (with metabolic activation). Evaluation of Results/Statistical Analyses - The test substance was classified as positive if it induced either a reproducible concentration-related increase in the mutant frequency or a reproducible positive response for at least one of the test points. A test substance producing neither a reproducible concentration-related increase in the mutant frequency nor a reproducible positive response at any of the test points is considered non-mutagenic in this system. As per OECD Guideline No. 476, no statistical evaluation of the data was performed.
Results:	Ambiguous
Remarks:	No precipitation of the test article was observed up to the maximal concentration evaluated. Experiment I: Mutagenic effects were observed but only at toxic concentrations without S9 mix (34.4 and 68.8 µg/mL) and with metabolic activation (825 µg/mlL. Experiment II: For confirmation of the results of the 1st experiment, a second was carried out. The dose range was adjusted to cover the critical dose range more closely and increasing doses were selected for a better demonstration of a possible dose-response relationship. In the second experiment mutagenicity was not observed, *i.e*. the findings of the first experiment were not reproduced. Relevant toxic effects were observed at 90 µg/mL and above in the absence and at 900 µg/mL and above in the presence of S9 mix. Experiment III (without S9 mix) and Experiment VI (with S9 mix): There was no increase in the mutation frequency up to the maximum doses tested either without S9 mix or after the addition of a metabolizing system.

	In the third experiment strong toxic effects were observed at 100 µg/mL without metabolic activation. In the sixth experiment relevant toxicity occurred at 150 µg/mL. At higher concentrations exceedingly strong toxic effects precluded the evaluation of results. According to the author the striking shift of toxicity in the presence of S9 mix may be based upon different batches of S9 used (differences in the content of lipids may be responsible, since whenever a test item binds to lipids shifts in toxicity are likely to occur because the concentration of free substance available to the cells is also different). The negative controls (untreated and vehicle) gave mutant frequencies within the range expected for the L5178Y cell line. Both positive controls showed a distinct increase in induced total mutant colonies and an increase of the relative quantity of small versus large colonies.
Conclusion:	Ethylbenzene gave an ambiguous result in the mouse lymphoma assay. The author considered ethylbenzene to be non-mutagenic in the mouse lymphoma assay since the effects observed in the first experiment were not reproduced in two additional experiments carried out independently of each other. The findings of the first experiment were thought to be caused by toxicity-related secondary effects and hence not indicative a true mutagenic potential of the test substance.
Data Quality:	3 – Not reliable. The protocol used in this assay is not a standard protocol. The 3-day expression period is considered suboptimal.
Reference:	Wollny, H.E. (2000). Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma cells (soft agar method) with ethylbenzene. RCC-CCR Project No. 635300. RCC-Cytotest Cell Research GmbH, Germany. Sponsored by the Styrenics Steering Committee, CEFIC, Brussels, Belgium.

[RS – 9]

Genetic Toxicity - *In Vitro* Gene Mutation: Mammalian Cell Mutation Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.85% supplied by Sigma, St. Louis, Missouri (Lot number: 01353MC)
Method:	OECD Guideline 476 – in Vitro Mammalian Cell Gene Mutation Test (1997) USEPA OPPTS 870.5300 (1998) EC, B.17 (2000)
Type:	Cell Mutation Assay at the Thymidine Kinase Locus (tk+/-)
System of Testing:	Mouse Lymphoma Cells L5178Y in culture
GLP:	Yes
Year:	2006
Species/Strain:	Mouse Lymphoma Cells L5178Y
Metabolic Activation:	With and Without
Species/cell type:	S9 liver homogenates prepared from Aroclor® 1254-induced male Sprague-Dawley rats, purchased from a commercial source.
Concentrations: **Tested** **Vehicle**	 10 to120 µg/mL (without S9) 10 to 120 µg/mL (with S9)
Remarks for Test Conditions:	The study investigated the potential of ethylbenzene to induce mutations at the thymidine kinase (tk) locus in L5178Y tk+/- mouse lymphoma cells using three experiments. <u>Assay A1- Preliminary Toxicity Assay</u> In a preliminary toxicity assay, the test material was evaluated at concentrations ranging from 4.2 to 1060 µg/mL in the absence and presence of metabolic activation system (S9). The highest concentration represents the limit dose of 10 mM and exceeded the solubility of the test material in the treatment medium. In the absence of S9, ethylbenzene was excessively toxic at the five highest concentration levels (i.e. 66.25, 132.5, 265, 530 and 1060 µg/mL) as measured by Day 2 relative suspension growth (RSG). The remaining cultures had day 2 RSG value ranging from 52 to 107%. In the presence of S9, excessive toxicity was observed at the 132.5 µg/mL concentration level and above as measured by Day 2 RSG. The remaining concentration levels had RSG values ranging from 15 to 95%. Based upon the results of this assay,

	concentrations in the range of 10 to 120 µg/mL were selected for the initial mutagenicity assay both in the absence and presence of S9. Assay B1 – Initial Mutagenicity Assay In the initial mutagenicity assay in the absence of S9, cultures treated with ethylbenzene showed excessive toxicity at concentration levels from 60 to 120 µg/mL as assessed by day 2 RSG and were not available for cloning. The remaining concentration levels (from 10 to 50 µg/mL) showed moderate to no toxicity and were selected for cloning. The relative total growth (RTG) in the test material treated cultures varied from 9 to 107%. In the presence of S9, cultures treated with ethylbenzene displayed excessive toxicity at concentrations of 80, 100 and 120 µg/mL. The RTG value in the remaining ethylbenzene treated (from 10 to 70 µg/mL) cultures varied from 21 to 104%. Assay C1- Confirmatory Mutagenicity Assay Based on the initial mutagenicity assay, the following concentrations were selected for the confirmatory mutagenicity assay – 10, 30, 38, 42, 46, 50, 54, 60 and 70 µg/mL in the absence of S9. Day 2 RSG values indicated excessive toxicity at 54, 60 and 70 µg/mL concentration levels. In the remaining cultures, day 2 RSG among these concentration levels, ranged from 15 to 99%. In the presence of S9, cultured treated with 90µg/ml of ethylbenzene displayed excessive toxicity as determined by day 2 RSG. Cultures treated with 82 µg/mL had individual RTG values of 9 and 10%. The mutant plates for these cultures were not enumerated since the average RTG value of the two replicates was <10% and the next two higher concentrations had RTG values in the acceptable range. Survival, as determined by day 2 RSG for the remaining cultures ranged from 9 to 100%. All other mutant plates from the remaining treatments were evaluated. All criteria for a valid assay, including positive responses in the positive control cultures were satisfied in this assay. Evaluation of Results/Statistical Analyses The test substance was considered positive when the average mutant frequency in at least one dose level of the treated cultures was 95 x 10^{-6} above the average of the concurrent solvent controls (assuming these to be in the range of 35-140 x 10^{-6}), or there was a positive dose related linear trend. This was tested using a linear trend test at alpha = 0.05, provided the above criterion was met. When there was no evidence of increase in mutant frequency at RTG values >10% the test substance was considered negative in this assay. The test substance was considered equivocal in this assay if there was a significant increase in mutant frequency that met the criteria for a positive response only at RTG values > 10% and < 20%, or there was no evidence of increase in mutant frequency at RTG values >20%. As per OECD Guideline # 476, no statistical evaluation of the data was performed.

Results:	Negative.
Remarks:	There was no increase in mutant frequency above 95 x 10^{-6}, the average of the concurrent controls, and there was no a positive dose related linear trend at any of concentrations of ethylbenzene evaluated in this assay with or without metabolic activation. Cultures treated with the positive control chemical induced a positive response as compared to the solvent control. The solvent control values were within the range of the laboratory historical data. All criteria for a valid assay were satisfied.
Conclusion:	Based upon results of the initial and confirmatory mutagenicity assays, ethylbenzene was considered to be non-mutagenic in the absence and presence of metabolic activation in this *in vitro* mouse lymphoma (L5178Y tk+/-) forward mutation assay.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data
Reference:	Seidel, S.D., Schisler, M.R. and Kleinert, K.M. (2006). Evaluation of Ethylbenzene in the Mouse Lymphoma (L5178YTK+/-) Forward Mutation Assay. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Study ID: 051157.

[RS - 10]

Genetic Toxicity - *In Vitro* Chromosome Aberrations

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	According to Galloway *et al*., *Environ. Mol. Mutagen* 10 (Suppl. 10): 1-175, 1987
Type:	Sister Chromatid Exchange Assay
System of Testing:	Chinese Hamster Ovary Cells
GLP:	Yes
Year:	1999
Species/Strain:	Chinese Hamster Ovary Cells
Metabolic Activation:	With and Without
Species/cell type:	Male Sprague-Dawley Rat / Liver; Aroclor 1254-induced (S-9 fraction)
Concentrations:	
Tested	75.5, 99.5, 125 µg/mL (without S9 mix) 125, 137.5, 150 µg/mL (with S9 mix)
Vehicle	DMSO
Remarks for Test Conditions:	Each test consisted of concurrent solvent and positive controls and 4 doses of ethylbenzene; the high dose was limited by toxicity. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. S9 was prepared from Aroclor 1254 induced male Sprague-Dawley liver. In the SCE test without S9, Chinese hamster ovary (CHO) cells were incubated for 26 hours with ethylbenzene in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing ethylbenzene was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 1.5 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the sister chromatid exchange (SCE) test with S9, cells were incubated with ethylbenzene, serum free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no ethylbenzene, and incubation proceeded for an additional 25.8 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides

	were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Evaluation of Results/Statistical Analyses - Statistical analyses were conducted on the slopes of the dose-response curves and individual data points. An SCE frequency of 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend in the absence of any responses reaching 20% above background was considered an equivocal response.
Results:	Negative
Remarks:	Dose(µg/mL) Relative Change of SCEs/Chromosome **-S9** 75.5 - 0.82 99.5 - 6.31 125 7.54 Mitomycin C 0.001 39.81 **+S9** 125 5.95 137.5 0.58 150 - 1.89 Cyclophosphomide 0.35 36.03
Conclusion:	Ethylbenzene did not induce sister chromatid exchanges in Chinese hamster ovary cells *in vitro*.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data
Reference:	National Toxicology Program. (1999). Toxicology and carcinogenesis studies of ethyl-benzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation studies) (Tech. Rep. Ser. No. 466; NIH Publ No. 99-3956), National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC

[RS - 11]

Genetic Toxicity - *In Vitro* Chromosomal Aberrations

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Not specified
Method:	According to Galloway *et al*., *Environ. Mol. Mutagen* 10 (Suppl. 10): 1-175, 1987
Type:	Cytogenetic Assay, Chromosomal Aberrations
System of Testing:	Chinese Hamster Ovary Cells
GLP:	Yes
Year:	1999
Species/Strain:	Chinese Hamster Ovary Cells
Metabolic Activation:	With and Without
Species/cell type:	Male Sprague-Dawley Rat / Liver; Aroclor 1254-induced (S-9 fraction)
Concentrations: **Tested** **Vehicle**	 75, 100, 125 µg/mL (without S9 mix) 75, 100, 125 µg/mL (with S9 mix) DMSO
Remarks for Test Conditions:	Each test consisted of concurrent solvent and positive controls and 4 doses of ethylbenzene; the high dose was limited by toxicity. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. S9 was prepared from Aroclor 1254 induced male Sprague-Dawley liver. In the chromosomal aberrations test without S9, cells were incubated in McCoy's 5A medium with ethylbenzene for 8.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberrations test with S9, cells were treated with ethylbenzene and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 8.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were then harvested in the same manner as for the treatment without S9. Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were

	scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and tranlocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations). Evaluation of Results/Statistical Analyses - Chromosome aberration data were presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual data points. For a single trial, a statistically significant difference for one dose point and a significant trend were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any dose level resulted in an equivocal call.
Results:	Negative
Remarks:	Compound / Dose (µg/mL) / Cells with Aberrations (%) **-S9** DMSO — 3 Ethylbenzene — 75 — 1 100 — 3 125 — 5 Mitomycin C — 1 — 22 **+S9** DMSO — 3 Ethylbenzene — 75 — 4 100 — 1 125 — 1 Cyclophosphamide — 50 — 36
Conclusion:	Ethylbenzene did not induce chromosomal aberrations in Chinese hamster ovary cells *in vitro.*
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1999). Toxicology and carcinogenesis studies of ethyl-benzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation studies) (Tech. Rep. Ser. No. 466; NIH Publ No. 99-3956), National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC

[RS –12]

Genetic Toxicity - *In Vitro* Micronucleus Assay

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99% supplied by the National Toxicology Program, USA
Method:	Experimental (Non – regulatory) - published in Kerckaert, GA., R.Brauninger, R.A. LeBoeuf and R.J. Isfort. (1996). Use of the Syrian hamster embryo cell transformation assay for carcinogenicity prediction of chemicals currently being tested by the National Toxicology Program in rodent bioassays. *Env. Health Perspectives.* 104(Suppl.5):1075-1084.
Type:	Induction of micronucleus in the Syrian hamster embryo (SHE) cell in vitro micronucleus assays
System of Testing:	Syrian Hamster Embryo Cells
GLP:	Not specified
Year:	1997
Species/Strain:	Syrian Hamster Embryo Cells
Metabolic Activation:	Without
Species/cell type:	Syrian Hamster Embryo Cell Culture
Concentrations: **Tested** **Vehicle**	 25,50,100 and 200 μg/mL DMSO
Remarks for Test Conditions:	The cells were seeded at $1x10^6$ cells/T-25 flask for control, and chemically-treated cultures. After approximately 24 hours, the cells were exposed to the test chemicals and cytochalasin B (3 μg/mL in DMSO) for 24 hours. The final concentration of solvent is approximately 1.3% (1% solvent +0.3% DMSO from the cytochalasin B). Colchicine (0.25 μg/mL or 0.5 μg/mL), or cyclophosphamide (5 μg/Ml) were used as positive controls. After a 24 hour treatment period, the media was aspirated off and the cells were collected by trypsinization. An aliquot of cells were counted to determine the number of live cells (determined by trypan blue exclusion) as a measure of toxicity. The remaining cells were suspended in 37^0C 0.075 M KCL for 5-10 minutes. The cells were collected by centrifugation and fixed in at least two changes of cold (4^O C) 25:1 methanol/acetic acid. The cells were then dropped on either dry or wet slides, air dried and stained for 1-5 minutes in a 10% Giemsa solution in Gurr buffer. At each treatment group, 500 cells were analyzed to determine the percentage of binucleated

	cells and 1000 binucleated cells were analyzed to determine the number of micronucleated cells. In case of ethylbenzene, fewer binucleated cells were scored due to a decrease in the number of scorable binucleated cells. Only cells with distinct cytoplasm and distinct binucleation were analyzed for the presence of micronuclei. Only micronuclei that were entirely inside the cytoplasm, separate from the main nucleus, less than approximately one-third the size of the main nuclei, and non-refractile were recorded. The number of micronucleated binucleated cells (MNBC): DMSO - 28/1000 25μg/mL - 54/1000 50μg/mL - 58/1000 100μg/mL - 71/1000 200μg/mL - 15/250
Results:	Positive
Remarks:	
Conclusion:	Ethylbenzene induced a significant increase in the frequency of micronucleated binucleated cells at all tested concentrations.
Data Quality:	2 – Reliable with restrictions. Study is non-GLP but sufficiently documented and meets generally accepted scientific principles.
Reference:	Gibson, D.P., Brauninger R., Shaffi H.S., Kerckaert G.A., LeBoeuf R.A.,Isfort R.J. and Aardema M.J. (1997). Induction of micronuclei in Syrian hamster embryo cells: Comparison to results in the SHE cell transformation assay for National Toxicology Program test chemicals. *Mutation Research* 392:61-70.

[RS - 13]

Genetic Toxicity - *In Vivo* Mammalian Erythrocyte Micronucleus

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99 %
Method:	
Type:	Micronucleus Assay (as described in MacGregor *et al*., *Fundam. Appl. Toxicol.* 14:513-522, 1990)
System of Testing:	Mouse, Peripheral Blood Erythrocytes
GLP:	Yes
Year:	1999
Species/Strain:	Mouse / B6C3F1
Sex:	Male and Female
#/sex/dose:	8 – 10
Route of Administration:	Inhalation
Doses/Concentrations:	500, 750, 1000 ppm – Vapor
Exposure Time:	6 hours/day, 5 days/week for 13 weeks
Remarks for Test Conditions:	Peripheral blood samples were obtained from male and female B6C3F1 mice at the end of the 13 week NTP toxicity study [see RS-17 for study details]. Smears were immediately prepared and fixed in absolute methanol, stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin & and coded. Slides were scanned to determine the frequency of micronuclei in 1000 polychromatic erythrocytes (PCEs) and 10000 normochromatic erythrocytes (NCEs) in each animal of each dose group. The criteria of Schmid (*Chemical Mutagens. Principles and Methods for their Detection,* A. Hollaender, Ed., Vol 4, pp.31-53. Plenum Press, New York 1976) were used to define micronuclei, with the additional requirement that the micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm and orange with 510 mn ultraviolet illumination); the minimum size limit was approximately one-twentieth the diameter of the NCE cell. In addition, the percentage of PCEs among the total erythrocyte population was determined. Evaluation of Results/Statistical Analyses - Log transformation of the NCE data, testing for normality by the Shapiro-Wilk test, and testing for heterogeneity of variance by Chochran's test were performed before statistical analyses. The frequency of

	micronucleated cells among NCEs was analyzed by analysis of variance using the SAS GLM procedure. The NCE data for each dose group were compared with the concurrent solvent control using Student's *t*-test. The frequency of micronucleated cells among PCEs was analyzed by the Cochran-Armitage trend test, and individual dose groups were compared to the concurrent solvent control by Kastenbaum-Bowman's binomial test. The percentage of PCEs among total erythrocytes was analyzed by an analysis of variance on ranks (classed by sex), and individual dose groups were compared with the concurrent solvent control using a *t*-test on ranks.
Results:	Negative
Remarks:	Dose (ppm) / Mean Micronucleated Cells/1000 cells (PCEs, NCEs) / PCEs (%) Male 0: 2.18, 1.54, 2.22 500: 2.04, 1.68, 3.13 750: 1.90, 1.90, 1.97 1000: 1.21, 1.59, 2.02 Female 0: 1.54, 0.92, 1.74 500: 2.64, 1.01, 1.83 750: 1.87, 1.32, 1.85 1000: 1.01, 1.12, 1.80
Conclusion:	Ethylbenzene did not induce micronuclei formation in bone marrow erythrocytes of mice following treatment up to the maximum tolerated concentration.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1999). Toxicology and carcinogenesis studies of ethyl-benzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation studies) (Tech. Rep. Ser. No. 466; NIH Publ No. 99-3956), National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC

[RS - 14]

Genetic Toxicity - *In Vivo* DNA Repair

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7%
Method:	OECD Guideline 486 - Unscheduled DNA Synthesis With Mammalian Liver Cells *In Vivo*
Type:	Unscheduled DNA Synthesis
System of Testing:	Mouse, Liver
GLP:	Yes
Year:	2001
Species/Strain:	Mouse / B6C3F1
Sex:	Male and Female
#/dose:	4 - 5 animals per sex per dose
Route of Administration:	Inhalation
Doses/Concentration:	Male mice – 500, 1000 ppm – Vapor Female mice – 375, 750 ppm – Vapor
Exposure Time:	6 hours
Remarks for Test Conditions:	Ethylbenzene was evaluated, using an autoradiographic technique, for its ability to induce unscheduled DNA synthesis (UDS) in the liver of B6C3F1 mice after a single 6 hour inhalation exposure (male mice: 500 and 1000 ppm; female mice 375 and 750 ppm). 1000 ppm and 750 ppm being the maximum tolerated concentration for each sex based on the patterns of clinical signs and lethalities observed over a 4 day period in a preliminary study. The concentration of ethylbenzene in generated atmosphere was determined at 8 sampling time points during the 6 hour exposure. The analytical chamber samples were analyzed using gas chromatography equipped with a flame ionization detector. A positive control, N-nitrosodimethylamine (N-DMA)(10 mg/kg bwt; oral) and a negative vehicle control group were run in parallel. Hepatocytes were isolated immediately after exposure, cultured in the presence of tritiated thymidine and subsequently examined for UDS following autoradiography. Evaluation of Results/Statistical Analyses - Data collected included the mean nuclear grain count, the mean cytoplasmic grain count,

	the mean net nuclear grain count, and the percentage of cells in repair. A mean net nuclear grain count greater than zero was considered to be indicative of a UDS response in that animal. No statistical analyses were applied.
Results:	Negative
Remarks:	The values recorded for the mean net nuclear grain counts and the percentages of cells in repair clearly show that ethylbenzene did not induce DNA repair, as measured by UDS, at any dose level investigated in either sex. The positive control induced marked increases in UDS compared to the vehicle control values. Dose (ppm) — Mean Net Nuclear Grain Count — Mean % Cells in Repair Male 0 - 6.1 0 500 - 6.6 1 1000 - 4.9 1 N-DMA (10 mg/kg) 13.1 79 Female 0 - 6.3 1 375 - 6.6 1 750 - 6.5 1 N-DMA (10 mg/kg) 24.3 89
Conclusion:	Ethylbenzene did not induce DNA repair (as measured by unscheduled DNA synthesis) in mouse liver following treatment up to the maximum tolerated concentration.
Data Quality:	1 – Reliable without restrictions. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Clay, P. (2001). Ethylbenzene: *In vivo* mouse liver unscheduled DNA synthesis assay. Central Toxicology Laboratory. CTL/SM0998/REG/REPT. Sponsored by the Styrenics Steering Committee, CEFIC, Brussels, Belgium.

[RS - 15]

Repeated Dose Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7 %
Method:	Not Specified
Type:	Repeated Dose Inhalation Toxicity Study
GLP:	Not specified but presumed Yes
Year:	1989
Species/Strain:	Rat / Fischer 344
Sex:	Male and Female
#/sex/dose:	5
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week, for 4 weeks
Doses/Concentrations:	100, 400, 800 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	<u>Animals and Maintenance</u> Species and strain: rat, Fischer 344 (Charles River Breeding Laboratories, Inc, Kingston, NY) Age on receipt: Approximately 6 weeks old Acclimation period: 17 days Housing: individually housed in suspended stainless-steel wire mesh cages Diet: Certified Rodent Chow No. 5002 (Purina), access *ad libitum* expect during the exposure period Water: access *ad libitum* expect during the exposure period Environment: Temperature averaged 72 ± 3 ºF and humidity ranged between 30 and 70% Body Weight at first treatment – Range between 158 – 178 g (males) and 124 – 138 g (females) <u>Chambers</u> Rats were exposed in common 10 m^3 stainless steel and glass inhalation chambers with mice and rabbits (results reported separately). Ethylbenzene concentrations in each chamber were

	monitored at hourly intervals, six times per day. Assessments Animals were observed twice daily for obvious clinical signs and underwent detailed examinations once each day after treatment. Body weights were measured weekly, and the weights of the major organs were recorded at termination. Ocular examinations were conducted on all animals immediately prior to exposure and at termination. Blood was collected via venipunture of the orbital sinus. Blood samples were subjected to hematological and clinical chemistry evaluation. Urinalyses were performed on all animals. At termination, animals were exsanginated under ether anesthesia. Over 30 tissues from each of the high-exposure and control animals were subjected to histopathological examination. Evaluation of Results/Statistical Analyses All parameters were evaluated for homogeneity of variance by Bartlett's test. When homogeneous, analysis of variance was conducted using the *F* distribution to assess significance. If the overall *F* statistic was significant, Dunnett's test was used to compare specific treatment groups to the control. The nonparametric Kruskall-Wallis test was used when variances were heterogeneous, and Dunn's summed-rank test was used to compare treated groups to controls.
Results:	NOAEL – 800 ppm, NOEL – 100 ppm (A NOAEL of 400 ppm was assigned by the author; however, the assignment was based on effects that the authors did not consider adverse, hence 800 ppm is the more appropriate study NOAEL and 100 ppm the study NOEL)
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 99, 382, and 782 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weight There were no treatment effects on survival or body weight gain. At 800 ppm, rats exhibited sporadic lacrimation and salivation. Clinical Pathology There were no treatment effects on clinical chemistry. Small but statistically significant increases in platelet counts occurred in male rats exposed to 800 ppm ethylbenzene. Also at 800 ppm, male rats showed a marginal increase and female rats a statistically significant increase in mean total leukocyte counts. The leukocytosis did not notably affect the differential white cell count. Pathology There were no treatment effects on gross and microscopic pathology. Exposure to 800 ppm resulted in an approximate 20% and 13% ($p < 0.01$) increase in relative (to body weight) liver weights in females and males, respectively. Female rats that received 400 ppm ethylbenzene exhibited about 7% increases ($p < 0.05$) in

	relative liver weights; whereas, the male relative liver weights were not significantly different from controls. The authors interpreted the liver changes as adaptive metabolic response due to the absence of liver histopathology or abnormal clinical chemistry.
Conclusion:	An increase in liver weight but no toxic effects were observed in rats that inhaled ≥ 400 ppm ethylbenzene vapor for 4 weeks. Also present at 800 ppm ethylbenzene were sporadic lacrimation and salivation and slight changes in blood cell counts.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Cragg, S.T., Clarke, E.A., Daly, I.W., Miller, R.R., Terrill, J.B. and Ouellette, R.E. (1989). Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fundam. Appl. Toxicol.* 13:399-408.

[RS - 16]

Repeated Dose Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7%
Method:	Not Specified
Type:	Repeated Dose Inhalation Toxicity Study
GLP:	Not specified but presumed Yes
Year:	1989
Species/Strain:	Mouse / B6C3F1
Sex:	Male and Female
#/sex/dose:	5
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week, for 4 weeks
Doses/Concentrations:	100, 400, 800 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	<u>Animals and Maintenance</u> Species and strain: mouse, B6C3F1 (Charles River Breeding Laboratories, Inc, Kingston, NY) Age on receipt: 7 weeks old Acclimation period: 17 days Housing: individually housed in suspended stainless-steel wire mesh cages Diet: Certified Rodent Chow No. 5002 (Purina), access *ad libitum* expect during the exposure period Water: access *ad libitum* expect during the exposure period Environment: Temperature averaged 72 ± 3 ºF and humidity ranged between 30 and 70% Body Weight at first treatment – Range between 19 – 24 g (males) and 17 – 21 g (females) <u>Chambers</u> Mice were exposed in common 10 m^3 stainless steel and glass inhalation chambers inhalation chambers with rats and rabbits (results reported separately).

	Assessments Animals were observed twice daily for obvious clinical signs and underwent detailed examinations once each day after treatment. Body weights were measured weekly, and the weights of the major organs were recorded at termination. Ocular examinations were conducted on all animals immediately prior to exposure and at termination. Blood was collected via venipunture of the orbital sinus. Blood samples were subjected to hematological evaluation. Clinical chemistry and urinalyses were not performed. At termination, animals were exsanginated under ether anesthesia. Over 30 tissues from each of the high-exposure and control animals were subjected to histopathological examination. Evaluation of Results/Statistical Analyses All parameters were evaluated for homogeneity of variance by Bartlett's test. When homogeneous, analysis of variance was conducted using the *F* distribution to assess significance. If the overall *F* statistic was significant, Dunnett's test was used to compare specific treatment groups to the control. The nonparametric Kruskall-Wallis test was used when variances were heterogeneous, and Dunn's summed-rank test was used to compare treated groups to controls.
Results:	NOAEL – 800 ppm, NOEL – 400 ppm (A NOAEL of 400 ppm was assigned by the author; however, the assignment was based on effects that the authors did not consider adverse, hence 800 ppm is the more appropriate study NOAEL and 400 ppm the study NOEL)
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 99, 382, and 782 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weights There were no treatment-related effects on survival, clinical signs, or body weight gain. Clinical Pathology There were no treatment-related effects on hematology. Pathology There were no treatment related effects on gross and microscopic pathology. In the mice that received 800 ppm ethylbenzene, liver weights relative to body weight were not statistically significantly different in males or females; but absolute liver weight was increased in females (about 15%; $p<0.05$), and liver weights relative to brain weights were increased in males (about 17%; $p< 0.05$) and females (about 15% at $p< 0.01$). The authors interpreted the liver changes as probably metabolic adaptation, due to the absence of liver histopathology or abnormal clinical chemistry.

Conclusion:	An increase in liver weight but no toxic effects were observed in mice that inhaled 800 ppm ethylbenzene vapor for 4 weeks.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Cragg, S.T., Clarke, E.A., Daly, I.W., Miller, R.R., Terrill, J.B. and Ouellette, R.E. (1989). Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fundam. Appl. Toxicol.* 13:399-408.

[RS - 17]

Repeated Dose Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7%
Method:	Not Specified
Type:	Repeated Dose Inhalation Toxicity Study
GLP:	Not specified but presumed Yes
Year:	1989
Species/Strain:	Rabbit / New Zealand White
Sex:	Male and Female
#/sex/dose:	5
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week, for 4 weeks
Doses/Concentrations:	400, 800, 1600 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	<u>Animals and Maintenance</u> Species and strain: rabbit, New Zealand White (Hazleton-Dutchland, Inc, Denver, CO) Age on receipt: Not specified Acclimation period: 6 weeks Housing: individually housed in suspended stainless-steel wire mesh cages Diet: Purina High-Fiber Rabbit Chow No. 5326 (Purina), access *ad libitum* expect during the exposure period Water: access *ad libitum* expect during the exposure period Environment: Temperature averaged 72 ± 3 ºF and humidity ranged between 30 and 70% Body Weight at first treatment – Range between 2934 – 3866 g (males) and 3034 – 3758 g (females) <u>Chambers</u> Rabbits were exposed in common 10 m^3 stainless steel and glass inhalation chambers inhalation chambers with rats and mice (results reported separately).

	Assessments Animals were observed twice daily for obvious clinical signs and underwent detailed examinations once each day after treatment. Body weights were measured weekly, and the weights of the major organs were recorded at termination. Ocular examinations were conducted on all animals immediately prior to exposure and at termination. Blood was collected via venipunture of the medial artery of the ear. Blood samples were subjected to hematological and clinical chemistry evaluation. Urinalyses were not performed. At termination, animals were overdosed with sodium pentobarbital. Over 30 tissues from each of the high-exposure and control animals were subjected to histopathological examination. In addition, testicular tissues from the low and intermediate exposure groups were also examined. Evaluation of Results/Statistical Analyses All parameters were evaluated for homogeneity of variance by Bartlett's test. When homogeneous, analysis of variance was conducted using the *F* distribution to assess significance. If the overall *F* statistic was significant, Dunnett's test was used to compare specific treatment groups to the control. The nonparametric Kruskall-Wallis test was used when variances were heterogeneous, and Dunn's summed-rank test was used to compare treated groups to controls.
Results:	NOAEL – 1600 ppm, NOEL – 800 ppm (A NOAEL of 800 ppm was assigned by the author; however, the assignment was based on effects that the authors did not consider adverse, hence 1600 ppm is the more appropriate study NOAEL and 800 ppm the study NOEL)
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 382, 782, and 1610 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weight There were no treatment-related effects on survival and clinical signs. During the first week of exposure, control rabbits showed less weight gain when compared to their gains over the remainder of the study, while 4 of the 6 ethylbenzene groups (except 800 ppm males and 400 ppm females) showed slight-to-moderate weight loss. This trend was more obvious in females than in males. Throughout the study, weekly weight gains in females were smaller in ethylbenzene exposed groups while differences in body weight were not statistically significant for females. After the first week of treatment, male rabbits in the ethylbenzene groups gained weight at the same rate as controls and had comparable body weights at the end of treatment. Clinical Pathology There were no treatment-related effects on clinical chemistry and hematology.

	Pathology There were no treatment-related effects on organ weights or gross and microscopic pathology.
Conclusion:	No toxic effects were observed in rabbits that inhaled up to 1600 ppm ethylbenzene vapor for 4 weeks.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Cragg, S.T., Clarke, E.A., Daly, I.W., Miller, R.R., Terrill, J.B. and Ouellette, R.E. (1989). Subchronic inhalation toxicity of ethylbenzene in mice, rats, and rabbits. *Fundam. Appl. Toxicol.* 13:399-408.

[RS - 18]

90-Day Subchronic Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99%
Method:	Generally meets the requirements of OECD Guideline 413: Subchronic inhalation Toxicity: 90-day study, with the following exceptions: feed consumption not measured, ophthalmic examinations not conducted, and adrenals not weighed.
Type:	Repeated Dose Inhalation Toxicity Study
GLP:	Yes
Year:	1992
Species/Strain:	Rat / Fischer 344/N
Sex:	Male and Female
#/sex/dose:	10
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week for 13 weeks
Doses/Concentrations:	100, 250, 500, 750, 1000 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	Animals and Maintenance Species and strain: rat, Fischer 344/N (Taconic Farms, Inc., Germantown, NY) Age on receipt: Approximately 5 weeks old Acclimation period: 12 days Housing: individually housed in suspended stainless-steel wire mesh cages Diet: NIH 07 Open Diet (Zeigler Bros., Inc.), access *ad libitum* expect during exposure Water: access *ad libitum* expect during the exposure period Environment: Temperature averaged 75 ± 3 ºF Age at first treatment: 7 weeks Chambers Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas

	chromatograph equipped with a flame ionization detector. Each study chamber atmosphere was analyzed at least once per hour during the 6 hour exposure. Animals and Treatment Groups of 10 rats of each sex were exposed to ethylbenzene for 6 hours per day, 5 days per week for 92 (female rats) and 93 (male rats) days. Controls were exposed to filtered air. Ten additional rats/sex were included at each exposure level to provide blood samples for clinical pathology (after blood collection on day 23, these rats were sacrificed and no tissues were retained). Assessments Blood for clinical chemistry and hematology was collected on study days 4 and 23, and at week 13 from the retroorbital sinus of male and female rats anesthetized with CO_2. Animals surviving to the end of the study were humanely sacrificed with CO_2. The heart, right kidney, liver, lung, right testis, and thymus were weighed. A necropsy was performed on all core study animals. Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for histopathological examination. Sperm morphology and vaginal cytology evaluations were performed according to methods described by Morrissey *et al*. (*Fundam. Appl. Toxicol.* 11:343-358, 1988). Evaluation of Results/Statistical Analyses Analysis of organ weight, serum chemistry, hematologic, and male reproductive system data was carried out to assess the significance of pair wise comparisons between dosed and chamber control groups, using nonparametric multiple comparison procedures. Jonckheere's test was used to evaluate the significance of dose-response trends to determine whether Dunn's or Shirley's test was more appropriate for pair wise comparisons.
Results:	NOAEL – 1000 ppm, NOEL – 100 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 99.4, 246, 498, 740, and 975 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weights There were no treatment-related deaths or clinical signs. Male and female rats at 1000 ppm had mild body weight depression (5-7%) which was not statistically significant. Clinical Pathology There were no treatment-related effects on hematology or clinical chemistry (except lower serum alkaline phosphatase). Serum alkaline phosphatase was decreased in a dose-related manner for both males (statistically significant, $p<0.05$, at $\geq$ 500 ppm) and female rats (statistically significant, $p<0.01$ at all dose levels). The significance of this decrease is not clear since, in liver damage,

	serum alkaline phosphatase levels usually increase. Pathology Absolute and/or relative weights of kidney, liver, or lung were higher at 250 ppm and greater ($p<0.05$ or $p<0.01$). At 1000 ppm, relative organ weight changes were 12, 29, and 18% in male kidney, liver and lung weights, respectively, and 17 and 29% in female liver and lung weights, respectively. There were no treatment-related histopathologic changes in any tissue. An enlargement of the bronchial and mediastinal lymph nodes was described at $\geq$ 250 ppm. Lymphoid hyperplasias of the bronchial and mediastinal lymph nodes were observed, as were inflammatory cell infiltrates around vessels, with foci of inflammatory cells in septae and lumen of alveoli in the lung. Although most rats were affected at exposure concentrations of 250 ppm and above, the severity of these lesions was not dose related. The occurrence and the severity of the inflammation corresponded to the observed increases in lung weights. The authors concluded that the inflammatory lung lesions in this study were probably unrelated to ethylbenzene exposure. There were no effects on sperm, testicular morphology, or estrus cycle. Since the organ weight changes occurred in the absence of hitopathological changes, these findings were not considered adverse and thus the NOAEL was considered to be 1000 ppm.
Conclusion:	Increases in liver and kidney weights but no toxic effects were observed in rats that inhaled $\geq$ 250 ppm ethylbenzene vapor for 13 weeks.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1992). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies). NTP Tox. 10. NIH Publication No., 92-3129. PB93-149722. National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC.

[RS - 19]

90-Day Subchronic Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	> 99%
Method:	Generally meets the requirements of OECD Guideline 413 - Subchronic inhalation Toxicity: 90-day study, with the following exceptions: feed consumption not measured, ophthalmic examinations not conducted, and adrenals not weighed.
Type:	Repeated Dose Inhalation Toxicity Study
GLP:	Yes
Year:	1992
Species/Strain:	Mouse / B6C3F1
Sex:	Male and Female
#/sex/dose:	10
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week for 13 weeks
Doses/Concentrations:	100, 250, 500, 750, 1000 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	<u>Animals and Maintenance</u> Species and strain: mouse, B6C3F1 (Taconic Farms, Inc., Germantown, NY) Age on receipt: approximately 5 weeks old Acclimation period: 15 days Housing: individually housed in suspended stainless-steel wire mesh cages Diet: NIH 07 Open Diet (Zeigler Bros., Inc.), access *ad libitum* expect during exposure Water: access *ad libitum* expect during the exposure period Environment: Temperature averaged 75 ± 3 ºF Age at first treatment: 7 weeks <u>Chambers</u> Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph equipped with a flame ionization detector. Each

	study chamber atmosphere was analyzed at least once per hour during the 6 hour exposure. Animals and Treatment Groups of 10 mice of each sex were exposed to ethylbenzene for 6 hours per day, 5 days per week for 97 (female mice) and 98 (male mice) days. Controls were exposed to filtered air. Assessments Animals surviving to the end of the studies were humanely sacrificed with CO_2. The heart, right kidney, liver, lung, right testis, and thymus were weighed. A necropsy was performed on all core study animals. Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for histopathological examination. Sperm morphology and vaginal cytology evaluations were performed according to methods described by Morrissey *et al*. (*Fundam. Appl. Toxicol.* 11:343-358, 1988). Evaluation of Results/Statistical Analyses Analysis of organ weight, serum chemistry, hematologic, and male reproductive system data was carried out to assess the significance of pair wise comparisons between dosed and chamber control groups, using nonparametric multiple comparison procedures. Jonckheere's test was used to evaluate the significance of dose-response trends to determine whether Dunn's or Shirley's test was more appropriate for pair wise comparisons.
Results:	NOAEL – 1000 ppm, NOEL – 500 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 99.4, 246, 498, 740, and 975 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weights There were no treatment-related deaths, clinical signs, or effects on body weight at termination. Pathology There was no treatment-related gross pathology findings at termination. There were dose-related higher absolute liver weights in males and females exposed at 750 and 1000 ppm ($p<0.01$), and a higher relative kidney weight in females at 1000 ppm ($p<0.05$). At 1000 ppm, the absolute liver weight increases were 25 and 28% in males and females, respectively, and the relative kidney weight was 11% higher in females. There were no treatment-related histopathologic findings in any organs. No differences from control were found in evaluation of sperm or vaginal cytology. Since the organ weights occurred in the absence of any histopathologic change, the NOAEL was considered to be 1000 ppm.

Conclusion:	Increases in liver and kidney weights but no toxic effects were observed in mice that inhaled ≥ 750 ppm ethylbenzene vapor for 13 weeks.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1992). Toxicity studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies). NTP Tox. 10. NIH Publication No., 92-3129. PB93-149722. National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC.

[RS - 20]

90-Day Subchronic Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.7%
Method:	OECD Guideline 408 – Repeated Dose 90-day Oral Toxicity Study in Rodents; US EPA Health Effects Test Guidelines. OPPTS 870.3100; EC Commission Directive 2001/59/EC of August 6, 2001, Part B
Type:	Repeated Dose Oral Toxicity Study
GLP:	Yes
Year:	2004
Species/Strain:	Rat / Wistar
Sex:	Male and Female
#/sex/dose:	10
Vehicle:	Corn Oil
Route of Administration:	Oral Gavage
Exposure Period and Frequency of Treatment:	Daily for 3 months Doses administered each day as 2 part doses with an interval of about 8 hours
Doses/Concentrations:	75, 250, 750 mg/kg bwt/day
Control Group:	Yes
Remarks for Test Conditions:	<u>Animals and Maintenance</u> Species and strain: rat, Wistar (CrlGlxBrlHan:WI)(Charles River, Sulzfeld, Germany) Age on receipt: approximately 34 days Acclimation period: not specified Housing: individually housed in suspended stainless-steel wire mesh cages Diet: ground Kliba maintenance diet mouse/rat “GLP” (Provimi Kliba SA, Kaiseraugst, Switzerland), access *ad libitum* expect during fasting period Water: access *ad libitum* expect during fasting period Environment: Temperature averaged 20-24 ºC; Relative Humidity averaged 30-70% <u>Dose Preparation</u>

	The concentrations of ethylbenzene preparations for all dose groups were analyzed. Assessments Feed and water consumption and body weights were determined weekly. The animals were examined for clinical signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out after 13 weeks of treatment. Clinicochemical, hematological examinations and urinalysis were performed towards the end of the administration period. Ophthalmological examinations were performed before and towards the end of the administration period. All animals were assessed by gross pathology, followed by histopathological examinations. Evaluation of Results/Statistical Analyses Analysis of body weight, body weight change, feed consumption, and feed efficiency was preformed by comparing treatment group results with the control group using Dunnett's test (two-sided) for the hypothesis of equal means. FOB, motor activity, clinical pathology parameters, and weight parameters, results were subjected to non-parametric one-way analysis using Kruskal-Wallis test (two-sided) followed by a Wilcoxon-test (two-side) if the p value was ≤ 0.05. Urinalysis data (except volume, color, turbidity, and specific gravity was analyzed by pair-wise comparison of each dose group with the control group using Fisher's exact test for the hypothesis of equal proportions.
Results:	NOAEL – 75 mg/kg bwt/day
Remarks:	Dose Confirmation The achieved concentrations of the test substance preparations for all dose groups were confirmed. The recovery rates at the study start were 96 to 99%. At the end, the recovery in the mid and high dose groups was 104 to 108%. At the low dose, a value of 227% was obtained so a reserved sample of this dose level was analyzed and gave a recovery rate of 87%. *All results are presented by target dosage level.* Mortality/Clinical Signs There were no treatment-related deaths. Clinical signs were post-dose salivation in ≥ 250 mg/kg bwt/day animals and discoloration of urine in 750 mg/kg bwt/day animals. Salivation may have been due to local irritation to the upper digestive tract. The urine finding was unexplained because no urine discoloration was seen during the urinalysis. Feed/Water Consumption and Body Weight 750 mg/kg bwt/day males exhibited an increase in feed consumption from day 70 onwards ($p<0.01$ or $p<0.05$), whereas body weight (13.8% below controls on day 91) and feed efficiency were decreased ($p<0.01$ or $p<0.05$). In females, body weight and feed efficiency were not affected by treatment. Water consumption

	was increased in 750 mg/kg bwt/day males (up to 47.6% above controls) and females (up to 45.9% above controls) and in 250 mg/kg bwt/day males with statistical significance achieved on several days ($p<0.01$ or $p<0.05$). Increased water consumption may indicate local irritation to the upper digestive tract. Neurologic Evaluation The value of the landing foot-splay test was significantly decreased in 750 mg/kg bwt/day males ($p<0.01$). The male mean foot-splay test values were 11.9, 11.9, 10.7 and 10.2 cm for the control, 75, 250, and 750 mg/kg bwt/day groups. This finding may have been related to the decrease body weight of this group. Rearing was significantly decreased in 75 mg/kg bwt/day females ($p<0.05$) but, due to the lack of a dose-response relationship, was considered incidental. The female mean rearing counts were 15.5, 10,8, 17.2, and 15.5 for the control, 75, 250, and 750 mg/kg bwt/day groups. Motor activity was significantly increased overall and at intervals 3, 6, and 7 in 750 mg/kg bwt/day females ($p<0.01$). The overall mean motor activity counts were 299.3, 350.9, 327.6, and 412.1 for the control, 75, 250, and 750 mg/kg bwt/day groups. The increases were atypical (usually an effect on motor activity is seen predominately either at the beginning or at the end of the measurement period) suggesting an incidental and not a treatment-related finding. Clinical pathology Enlarged mean corpuscular volume was observed in 750 mg/kg bwt/day animals of both sexes ($p<0.01$) and 250 mg/kg bwt/day females ($p<0.05$). Decreased platelet counts were present in 750 mg/kg bwt/day females ($p<0.01$). Both findings were considered treatment-related and possibly due to a minimal transitional regenerative anemia. Prothrombin times were shorter in $\geq$250 mg/kg bwt/day animals of both sexes ($p<0.01$or $p<0.05$), however, the reduction of prothrombin times in 750 mg/kg bwt/day males occurred only as a tendency towards shorter clotting times. Slight increases in alanine aminotransferase ($p<0.01$) and γ-glutamyltransferase ($p<0.01$) were found in sera of $\geq$ 250 mg/kg bwt/day males at the end of the study. In females, alanine aminotransferase activity was increased only in 750 mg/kg bwt/day animals ($p<0.05$). Increased potassium, total bilirubin, albumin, cholesterol and magnesium concentrations were present in 750 mg/kg bwt/day animals of both sexes ($p<0.01$ or $p<0.05$). In addition, increased calcium and urea and decreased creatinine concentrations were found in 750 mg/kg bwt/day males ($p<0.01$ or $p<0.05$) and increased potassium, calcium, total bilirubin and cholesterol were noted in 250 mg/kg bwt/day males ($p<0.01$ or $p<0.05$). ($p<0.01$ or $p<0.05$). In females, total protein and globulin concentrations were increased and sodium decreased in 750 mg/kg bwt/day animals ($p<0.01$ or $p<0.05$). Higher cholesterol values were also noted in $\geq$ 250 mg/kg bwt/day animals ($p<0.01$). The clinical chemistry changes were speculated to be due to induction of the hepatic microsomal enzyme system and/or secondary to effects on feed and water consumption. Urine analysis Increased numbers of degenerated transitional epithelial cells and

	granular epithelia cell casts were present in ≥ 250 mg/kg bwt/day males (p<0.01 or p<0.05). The findings indicated mild damage or functional impairment to the kidneys. Pathology Compound-related changes occurred in the liver of both sexes and in the male kidney. In the liver, an increase in absolute and relative weights was recorded in both sexes at ≥ 250 mg/kg bwt/day (p<0.01). At 750 mg/kg bwt/day, the relative liver weights were increased 47 and 34% in males and females, respectively. These liver weight increases were correlated with an accompanying centribolular hypertrophy of hepatocytes in the majority of animals, indicating an adaptive, reactive response in the liver. No compound-related toxic structural changes were detected in the liver. In 75 mg/kg bwt/day animals, a very slight increase (5%) in relative liver weights in the males was recorded but was not accompanied by histopathological change and hence considered of no biological relevance. In the kidneys of ≥ 250 mg/kg bwt/day males, an increase in absolute and relative weights was noted (p<0.01). At 750 mg/kg bwt/day, male relative kidney weights were increased 35%. The only histopathologic finding attributed to treatment was an increase of hyaline droplet storage in the tubular epithelium. This lesion correlated with the absolute weight increase in the male kidney only. The increase in hyaline droplets was considered as an increase of the male specific protein α-2u-globulin. Morphologic signs of Chronic Progressive Nephropathy were similar in the control and treatment groups. Females that received ≥ 250 mg/kg bwt/day ethylbenzene exhibited slight increases in relative kidney weight (8-13%)(p<0.01) that was not correlated with histopathological changes and hence was considered of no biological relevance. A significant decrease of absolute and relative (16%) thymus weights (p<0.01 or p<0.05) were present in ≥ 250 mg/kg bwt/day females but were not correlated with histopathological changes and hence was considered of no biological relevance.
Conclusion:	Changes in hematology, indicative of a mild regenerative anemia, and clinical chemistry parameters, indicative of hepatic microsomal enzyme induction, decreases in prothrombin time, mild alimentary effects and kidney (males only) and liver pathology were observed in rats that received gavage doses of ≥ 250 mg/kg bwt/day ethylbenzene for 90 days.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Mellert, W., Deckardt, K., Kaufmann, W., and van Ravenzwaay, B. (2004). Subchronic toxicity study in Wistar rats. Oral Administration by gavage for 3 months. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Laboratory Project Identification Project No. 50C0153/99153. Sponsored by the Styrenics Steering Committee, CEFIC, Brussels, Belgium.

[RS - 21]

Reproduction and Fertility Effects

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.0%
Method:	Pilot Study for a 2-Generation Reproductive Toxicity Study
Type:	Pilot Reproductive Toxicity Study
GLP:	Yes
Year:	2003
Species/Strain:	Crl:CD® (Sprague-Dawley) IGS BR
Sex:	Male and Female
#/sex/dose:	20
Vehicle:	Air (Inhalation) Corn Oil (Gavage)
Route of Administration:	Inhalation Vapor Gavage (select females, lactation days 1 through 4)
Exposure Period and Frequency of Treatment:	**F_0** : Inhalation vapor – Daily, 6 hour-exposures; males exposed to test atmospheres for a minimum of 4 weeks. Females were exposed to test atmospheres for a minimum of 2 weeks prior to mating, throughout the mating period, and from gestation day 0 through gestation day 20. At this time, one-half of the F_0 females were selected for the inhalation/gavage phase and the remaining females selected for the inhalation phase only. Inhalation exposure of the F_0 females was suspended from gestation day 21 through lactation day 4. Inhalation exposure of the F_0 females in both phases was re-initiated on lactation day 5 and continued through the day prior to euthanasia. Oral gavage - The inhalation/gavage females received the vehicle, corn oil, or the test article in the vehicle via oral gavage at dose levels of 0, 90, 342, and 621 mg/kg bwt/day (divided into 3 equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg bwt /dose during lactation days 1 through 4. **F_1 -** The F_1 offspring were potentially exposed to the test article *in utero* and through nursing during lactation until weaning. On lactation days 21 and 28, pups were weaned and selected (one pup/sex/litter) for inhalation exposure to the same concentration of the test article as their dam beginning on postnatal day 22 or 29 and lasting through postnatal day 33.

Doses/Concentrations:	Inhalation vapor – 100, 500, 1000 ppm Oral gavage – 90, 342, 621 mg/kg bwt/day (30, 114, 207 mg/kg bwt/day, respectively, administered three times per day at approximately 2 hour intervals) at 1 mL/kg bwt/dose
Control Group:	Inhalation – Air Gavage – Corn oil
Statistical Methods:	Chi-square test with Yates' correction factor: parental mating and fertility indices Parametric one-way analysis of variance (ANOVA) for homogeneous and normal data or Kruskal-Wallis nonparametric ANOVA for non-homogeneous or non-normal data: mean parental (weekly, gestation and lactation) and F_2 offspring body weight, food consumption and food efficiency data, organ weight data, maternal estrous cycle data, pre-coital intervals, gestation lengths, implantation sites, unaccounted sites, numbers of pups born, and live litter size. Kruskal-Wallis nonparametric ANOVA: mean litter proportions (percent per litter) of postnatal pup survival, sex ratio at birth (percentage of males per litter. Nested analysis of covariance (ANCOVA): pup weights through weaning
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Crl:CD (SD)IGS BR (Charles River Laboratories, Raleigh, NC) - Age: 56 days on receipt - Acclimation period: 16 days - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum* except during exposure - Water: reverse osmosis-treated tap water, *ad libitum* except during exposure Environment: controlled to 71 ± 5 ºF, 50 ± 20% relative humidity, 12 hour light/dark cycle, 10 air changes/hour - F_0 age at first treatment: approximately 11 weeks - F_0 bwt at first treatment: males = 341-406 g, females = 225-273 g <u>Chamber Conditions</u> Rats were exposed in 2 m^3 stainless steel and glass whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured at least 10 times per exposure day by an automatic sampling system coupled to a gas chromatograph. <u>Treatment</u> Four groups of male and female Crl:CD®(SD)IGS BR rats (20/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article. Target test article concentrations were 100, 500 and 1000 ppm for the F_0 generation and selected F_1 weanlings.

	F_0 males were exposed to test atmospheres for a minimum of 4 weeks F_0 females were exposed to test atmospheres for a minimum of 2 weeks prior to mating, throughout the mating period, and from gestation day 0 through gestation day 20. At this time, one-half of the F_0 females were selected for the inhalation/gavage phase and the remaining females selected for the inhalation phase only. Inhalation exposure of the F_0 females was suspended from gestation day 21 through lactation day 4. The inhalation/gavage females received the vehicle, corn oil, or the test article in the vehicle via oral gavage at dose levels of 0, 90, 342, and 621 mg/kg/ bwt/day (divided into 3 equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg bwt /dose during lactation days 1 through 4. Inhalation exposure of the F_0 females in both phases was re-imitated on lactation day 5 and continued through the day prior to euthanasia. The F_1 offspring were potentially exposed to the test article *in utero* and through nursing during lactation until weaning. On lactation days 21 and 28, pups were weaned and selected (one pup/sex/litter) for exposure to the same concentration of the test article as their dam beginning on postnatal day 22 or 29 and lasting through postnatal day 33. <u>Preparation of Dosing Solutions</u> Oral dosing solutions were prepared weekly in corn oil vehicle and stored at room temperature for a period not to exceed 1 week in duration. <u>Analysis of Dosing Solutions</u> An aliquot from each formulation was taken on the first day of dose administration and once during the gavage exposures and analyzed by GC. Stability was determined over 10 days (room temperature). <u>Mating</u> Animals (20 per sex/group) were paired in a 1:1 basis after treatment for at least 2 weeks. Each pair was examined daily and mating was confirmed by the presence of a copulatory plug or presence of sperm in a vaginal smear (assigned gestation day 0). If no evidence of mating was apparent after 14 days, the female was placed in a plastic maternity cage with nesting material. F_0 bwt prior to mating: males 339-443 g, females 222-301 g; approximate age 13 weeks. <u>F_0 and F_1 Observations</u> All animals were observed twice daily (prior to exposure and within 1 hour after completion of each exposure period) for external clinical signs, behavioral changes and mortality, and subject to a more detailed physical examination once weekly. Male body weight and food intake were recorded weekly until euthanasia. Female body weight and feed consumption were recorded weekly until mating, on 8 occasions during gestation (3 day intervals) and 7 occasions during lactation (3-7 day intervals). <u>Litter Observations</u> Each litter was examined daily for survival. A detailed gross

	necropsy was conducted on any pup dying from postnatal days 0 to 4 to prior to weaning. Litter Reduction On postnatal day 4, litter size was reduced to 10 pups per litter (5/sex where possible) to reduce variability among litters. Pup Parameters Each pup was subject to a detailed physical examination on postnatal days 1, 4, 7, 14, 21 and 28. Pup body weights were recorded on postnatal days 1, 4, 7, 14, 21 and 28. Weaning and Selection Each dam and litter remained housed together until weaning on lactation day 28 except for one pup/sex/litter that was weaned on postnatal day 21. The day following weaning (postnatal day 21 or 28), one male and one female per litter that were randomly selected were directly exposed to the same concentration of the test article as their dam through postnatal day 33. Direct inhalation exposure periods for the selected pups were postnatal days 22-33 (F_1 postnatal days 22-33) or postnatal days 29-33 (F_1 postnatal day 29-33). Necropsy - Adults All parental animals were subject to gross examination (including any unscheduled deaths). For females that delivered the number of former implantation sites were recorded. For females that failed to deliver, nongravid uteri were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Organ weights were recorded for livers and kidneys Necropsy - Pups A gross necropsy was performed on all pups.
Results:	Parental Toxicity NOAEL – 1000 ppm or 1000 ppm/642 mg/kg bwt/day, NOEL – 100 ppm or 100 ppm/90 mg/kg bwt/day Reproductive Toxicity NOAEL – 1000 ppm or 1000 ppm/642 mg/kg bwt/day Developmental Toxicity NOAEL – 100 ppm or 100 ppm/90 mg/kg bwt/day
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers for the F_0 generation were 99, 500, and 1008 ethylbenzene. Actual mean exposure concentrations achieved in the chambers for the F_1 generation were 101, 498, and 1002 ethylbenzene. Analysis of Dosing Solutions Dosing formulations were analyzed demonstrating the formulations were homogeneous and stable (up to 10 days). Results of periodic concentration analyses returned the following mean concentration ranges:

	-----mg/mL----- Target Actual 30 29.9 – 33.7 114 112 – 122 207 205 – 231 *All results are presented by target inhalation or dosage level.* Survival and Clinical Signs There were no test article-related clinical observations or deaths in F_0 animals. Several exposure-related deaths were observed in the F_1 500 and 1000 ppm group (inhalation phase) weanlings and the F_1 1000 ppm/621 mg/kg bwt/day (inhalation/gavage) phase weanlings that initiated exposure on postnatal day 22 (postnatal days 22-33 animals). Exposure-related clinical signs observed one hour following dosing included labored respiration, eyelids half-closed, prostrate, righting difficulty, rocking, lurching, and swaying while ambulating. These findings were generally noted in the first several days of exposure only. No adverse effects were observed on clinical signs in the F_1 weanlings exposed to 100 ppm of ethylbenzene beginning on postnatal day 22. No deaths or exposure-related clinical signs were noted in the F_1 weanlings exposed to ethylbenzene beginning on postnatal day 29 (postnatal days 29-33). No adverse effects were observed on clinical signs in the F_1 weanlings exposed to 100 ppm of ethylbenzene beginning on postnatal day 29. Body Weight and Feed Intake F_0 Body weight gain was decreased in the 500 and 1000 ppm group males and females during the first week of exposure ($p<0.05$ or $p<0.01$) and continued to be reduced in the 1000 ppm group males during the second week of exposure (not statistically significant). As a result of the reduced body weight gain during the first 2 weeks of exposure, mean body weights in the 1000 ppm group F_0 males were reduced 4.2-4.7% during study weeks 2-4 ($p<0.05$). In the F_0 females the reduction was not of sufficient magnitude to produce a reduction in mean body weight relative to control. Body weight parameters were unaffected in the low exposure group. Mean body weight gain was reduced in the F_1 weanlings exposed to 500 and 1000 ppm beginning on postnatal day 22 in both the inhalation and inhalation/gavage phases (postnatal days 22-33 animals)($p<0.05$ or $p<0.01$). Reductions in mean body weights ranged from 3-14% in the 500 ppm animals and 9-27% in the 1000 ppm animals. Slightly reduced mean body weights were noted in the F_1 weanlings exposed to 100 ppm beginning on postnatal day 22 in both the inhalation (4-14%) and inhalation/gavage (6-16%) phases. The only statistically significant difference from the control group was noted on postnatal day 31 for females in the inhalation phase. Postnatal days 29-33 animals exhibited reduced mean body weight gain at 500 or 1000 ppm in both the inhalation and inhalation/gavage phases. The differences from the control group were statistically

	significant ($p<0.05$ or $p<0.01$) for the inhalation only animals. At 1000 ppm by postnatal day 34, body weights were reduced by 6–13% and, at 500 ppm by postnatal day 34, body weights were reduced by 5-9%. Mean body weights and body weight gains in the F_1 weanlings exposed to 100 ppm of ethylbenzene beginning on postnatal day 29 were similar to control group values. Reproductive Performance Ethylbenzene did not adversely affect reproductive performance in either sex. (by dose group: 0, 100, 500, 1000 ppm) Mating index (%) - F_0 males: 100, 100, 100, 95 - F_0 females: 100, 100, 100, 95 Fertility index (%) - F_0 males: 75, 85, 100, 95 - F_0 females: 75, 85, 100, 95 Mean pre-coital interval (days) - F_0: 3.4, 2.7, 3.2, 2.4 Gestation Length The mean length of gestation was unaffected by treatment. (by dose group: 0, 100, 500, 1000 ppm)(days) F_0: 22.1, 21.8, 21.9, 21.8 Necropsy Observations At the scheduled necropsies, no macroscopic findings related to test article exposure were observed at any exposure concentration. The mean number of implantation sites did not differ between the groups: (by dose group: 0, 25, 100, 500, 1000 ppm) - F_0: 15.9, 15.5, 15.7, 15.5 Organ Weights (F_0) Mean liver (males and females) and kidney (males only) weights were increased in the 500 and 1000 ppm (generally statically significant, $p<0.01$). Relative liver weights were increased 13.1 and 26.6% in the 500 and 1000 ppm male groups, respectively. Relative kidney weights were increased 10.5 and 19.0% in the same respective groups. In females, relative liver weights were increased 21.9 and 37.6% at 500 and 1000 ppm, respectively, inhalation phase only and 14.0 and 25.8 for these same groups for inhalation/gavage phase (generally statically significant, $p<0.05$ or $p<0.01$). Litter Data No statistically significant differences were present in litter parameters. Combined Phase By dose group: 0/0, 100/90, 500/342 and 1000/621 ppm/mg/kg bwt/day

	Live litter size: - F_1: 14.2, 14.5, 13.8, 13.7 No. live pups: - F_1: 14.3, 14.7, 14.6, 14.4 Males/litter (%): - F_1: 48.5, 48.1, 46, 49 <u>Pup Survival</u> Postnatal survival was slightly reduced from birth to postnatal day 4 in the 500 and 1000 ppm groups (inhalation phase) and in the 1000 ppm/621 mg/kg bwt/day group (inhalation/gavage phase): By dose group: 0/0, 100/90, 500/342, 1000/621 ppm/mg/kg bwt/day From birth to postnatal day 4 (pre-selection): (%) - F_1 inhalation phase: 99.1, 91.7, 86.5, 84.1* - F_1 Inhalation/gavage phase: 95.1, 97.2, 94.6, 88.7 (* not statistically significantly different but lower than concurrent and historical control data and occurred in presence of other adverse effects to pups, hence considered treatment-related) <u>Pup body weight:</u> Pup weight reductions occurred in the 1000 ppm inhalation exposure groups and were more pronounced in the inhalation/gavage phase (14.8 and 10.9% in male and female pups, respectively, on postnatal day 28)($p<0.05$ or $p<0.01$) than in the inhalation phase (3.4 and 4.3% in the male and female pups, respectively, on postnatal day 28) during the pre-weaning period. These results suggest that the gavage dosing of the dams on lactation days 1 to 4 affected the growth of the offspring. <u>Pup Necropsy Findings</u> No treatment-related changes were apparent in the pups.
Conclusion:	Ethylbenzene at an exposure level of 1000 ppm did not adversely impact reproduction but at ≥ 500 ppm (340 mg/kg bwt/day) produced developmental toxic effects and toxicity in exposed weanling rats.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Stump, D.G. (2003). A pilot inhalation study for a reproductive toxicity study of ethylbenzene in rats. Study Number – WIL-186028, WIL Research Laboratories, Inc., Ashland, OH. Sponsored by the Ethylbenzene Panel, American Chemistry Council, Arlington, VA.

[RS - 22]

Reproduction and Fertility Effects

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.9%
Method:	EPA Health Effects Testing Guidelines OPPTS 870.3800 and OECD Guideline 416 – Two-Generation Reproductive Toxicity Study
Type:	Two Generation Reproductive Toxicity Study
GLP:	Yes
Year:	2004
Species/Strain:	Crl:CD® (Sprague-Dawley) IGS BR
Sex:	Male and Female
#/sex/dose:	F_0 Generation - 30 F_1 Generation - 25
Vehicle:	Air (Inhalation) Corn Oil (Gavage)
Route of Administration:	Inhalation Vapor Oral Gavage (females, lactation days 1 through 4)
Exposure Period and Frequency of Treatment:	**F_0:** Inhalation vapor - Daily 6-hour exposures; males exposed for 70 consecutive days prior to mating and through the day prior to euthanasia; females exposed for 70 consecutive days prior to mating, through gestation day 20 and from lactation day 5 through the day prior to euthanasia Oral gavage (females that delivered only) - Daily doses for 4 consecutive days (lactation days 1 to 4) **F_1**: Inhalation vapor - F_1 offspring potentially exposed *in utero* and through nursing during lactation; daily 6-hour exposures after weaning (postnatal day 21); males exposed for a minimum of 70 consecutive days prior to mating and through the day prior to euthanasia; females exposed for a minimum of 70 consecutive days prior to mating, through gestation day 20 and from lactation day 5 through the day prior to euthanasia Oral gavage (females that delivered only) - Daily doses for 4 consecutive days (lactation days 1 to 4) **F_2**: F_2 offspring potentially exposed *in utero* and through nursing during lactation

Doses/Concentrations:	Inhalation vapor: 25, 100 and 500 ppm Oral gavage: 26, 90, and 342 mg/kg bwt/day (8.87, 30, and 114 mg/kg bwt/day, respectively, administered three times per day at approximately 2-hour intervals) at 1 mL/kg bwt dose
Control Group:	Air (Inhalation) Corn Oil (Gavage)
Statistical Methods:	Chi-square test with Yates' correction factor: parental mating and fertility indices Parametric one-way analysis of variance (ANOVA) for homogeneous and normal data or Kruskal-Wallis nonparametric ANOVA for non-homogeneous or non-normal data: mean parental (weekly, gestation and lactation) and F_2 postweaning offspring body weight, feed consumption and feed efficiency data, organ weight data, maternal estrous cycle data, pre-coital intervals, gestation lengths, implantation sites, unaccounted sites, ovarian primordial follicle counts, numbers of pups born, live litter size, epididymal and testicular sperm numbers, sperm production rates and F_2 day of acquisition of pre-weaning/post-weaning developmental landmarks Kruskal-Wallis nonparametric ANOVA: mean litter proportions (percent per litter) of postnatal pup survival, sex ratio at birth (percentage of males per litter), percentage of motile and progressively motile sperm and percentage of sperm with normal morphology Nested analysis of covariance (ANCOVA): pup weights through weaning Two-tailed Fisher's Exact test: histopathological findings
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Crl:CD (SD)IGS BR (Charles River Laboratories, Raleigh, NC) - F_0 age on receipt: 38 days - Acclimation period: 13 days - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum* except during exposure - Water: reverse osmosis-treated tap water, *ad libitum* except during exposure Environment: controlled to 71 ± 5 ºF, 50 ± 20% relative humidity, 12 hour light/dark cycle, 10 air changes/hour - F_0 age at first treatment: approximately 51 days - F_1 age at first treatment: 22 days <u>Chamber Conditions</u> Rats were exposed in 2 m^3 stainless steel and glass whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured at least 10 times per exposure day by an automatic sampling system coupled to a gas chromatograph.

	Treatment Four groups of male and female Crl:CD®(SD)IGS BR rats (F_0 generation: 30/sex/group; F_1 generation: 25/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, for 6 hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 0, 25, 100 and 500 ppm (parts per million) for the F_0 and F_1 generations. Exposures were initiated when the F_0 animals were approximately 7 weeks of age and the F_1 animals were 22 days of age. Inhalation exposure of the F_0 and F_1 males continued throughout mating, and through the day prior to euthanasia. The F_0 and F_1 females continued inhalation exposure throughout mating and gestation through gestation day 20. Inhalation exposure of the F_0 and F_1 females was suspended from gestation day 21 through lactation day 4 since prolonged maternal separation from litters at this early postnatal stage was considered too stressful for the neonates. Therefore on lactation days 1 through 4, the F_0 and F_1 females received the corn oil vehicle or test article in the vehicle via oral gavage at dose levels of 0, 26, 90 and 342 mg/kg bwt/day (divided into three equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg bwt/dose. Inhalation exposure of the F_0 and F_1 females was re-initiated on lactation day 5 and continued through the day prior to euthanasia. For reporting purposes, F_0 and F_1 male inhalation exposure groups and all groups for the F_2 generation component of the study were designated as 0 ppm, 25 ppm, 100 ppm and 500 ppm. Inhalation/gavage exposure group designations for F_0 and F_1 females were 0 ppm/0 mg/kg bwt/day, 25 ppm/26 mg/kg bwt/day, 100 ppm/90 mg/kg bwt and 500 ppm/342 mg/kg bwt/day. Preparation of Dosing Solutions Oral dosing solutions were prepared weekly in corn oil vehicle and stored at room temperature for a period that did not exceed 10 days in duration. Analysis of Dosing Solutions An aliquot from each formulation was taken from each weekly preparation and analyzed by GC. Stability was determined over 10 days (room temperature). Mating Daily vaginal lavages were performed for determination of estrous cycles, beginning 21 days prior to pairing (avoiding sibling pairings). Animals (30 per sex per dose group for F_0 and 25 per sex per dose group for F_1 generations) were paired in a 1:1 basis (adjustments were made only to avoid sibling pairings) after treatment for at least 70 days. Each pair was examined daily and mating was confirmed by the presence of a copulatory plug or presence of sperm in a vaginal smear (assigned gestation day 0). Females with no evidence of mating after 14 days (or three estrus cycles) were paired with another male of the same exposure group (avoiding sibling pairing) that had successfully mated for an additional 7 days. If no evidence of copulation was obtained after 21 days, the female was placed in a plastic maternity cage with nesting material. F_0 bwt prior to mating (week 10; age approx. 17 weeks): males 399-610 g, females 241-386 g. F_1 bwt prior to mating (week 27; age approx. 15

	-16 week): males 311-590 g, females 244-370 g. F_0 and F_1 Observations All animals observed twice daily for external clinical signs, behavioral changes and mortality, and subject to a more detailed physical examination once weekly. Male body weight and feed intake were recorded weekly until euthanasia. Female body weight and feed consumption were recorded weekly until mating, on 6 occasions during gestation (3 to 6 day intervals) and 6 occasions during lactation (3 to 7 day intervals). The stage of estrus (metestrus, diestrus, estrus, proestrus) for each female (vaginal smear) was recorded commencing from 21 days prior to mating and continuing until mating was confirmed. Litter Observations Each litter was examined daily for survival. Any pups dying on postnatal days 0 to 4 were subject to necropsy (including examination of heart and brain plus skeletal examination if hard tissue anomaly suspected). A detailed gross necropsy was conducted on any pup dying between postnatal day 4 and prior to weaning, and tissues preserved for histological examination. Litter Reduction On postnatal day 4, the litters from both generations (F_1 and F_2 pups) were reduced to 10 pups per litter (5/sex, where possible) to reduce variability among litters. Pup Parameters Each pup was subject to a detailed physical examination on postnatal days 1, 4, 7, 14 and 21. Anogential distance was measured on postnatal day 1, and pups individually sexed on postnatal days 0, 4, 7, 14 and 21. F_1 body weights were recorded on postnatal days 1, 4, 7, 14 and 21. F_2 body weights were recorded on postnatal day 1, 4, 7, 11, 13, 17, and 21. Developmental Landmarks Each pup was evaluated for pinnal detachment beginning on postnatal day 4 and continuing until both auricles of the pinnae were fully detached or until scheduled euthanasia. Each pup was evaluated for incisor eruption beginning on postnatal day 7 until both upper and lower incisors had erupted. Each pup was observed for normal hair growth beginning on postnatal day 8 until hair growth was considered normal. Each pup was evaluated for eyelid separation beginning on postnatal day 12 until both eyelids were fully open or until scheduled euthanasia. Balanopreputial separation was assessed in males (30 per dose group for F_1 males and 20 dose group for F_2 males) from postnatal day 35 onwards. Vaginal opening was assessed in females (n = 30 per dose group) from postnatal day 25 onwards. Body weight was recorded for all pups on the day of acquisition of each developmental landmark. Selection of F_1 Parents On postnatal day 21, two F_1 male and two F_1 females weanlings per litter were selected and exposed to test article for 6 hours/day beginning on day 22 *post partum*. Of these weanlings, 25 male and 25 female F_1 pups from each group were randomly selected on

	postnatal day 28 to comprise the F_1 generation. Necropy - Adults All F_0 and F_1 parental animals were subject to examination (including any unscheduled deaths). For females that delivered the number of former implantation sites were recorded. For females that failed to deliver, nongravid uteri were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Organ weights were recorded for all major organs, including pituitary, thymus, thyroid, epididymides (total and cauda), ovaries, prostate, seminal vesicles (with coagulating glands and accessory fluids), testes and uterus with oviducts and cervix. An extensive range of tissues were sampled and preserved as per test guidelines. Ovarian primordial follicle counts were recorded for all control and 750 ppm F_1 females and for 25 and 100 ppm F_1 females that failed to mate or produce offspring. Evaluation of Spermatogenic Endpoints The right epididymis was excised immediately upon euthanasia, weighed and a sample of sperm collected from the right cauda epididymis for assessment of: - motility (200 motile and nonmotile spermatozoa per animal, all dose groups, Hamiliton-Thorne HTM-IVOS Version 10 computer assisted sperm analysis system) - morphology of abnormal forms evaluated by differential microscopic count of 22 spermatozoa per animal light using wet-mount technique (Linder *et al. Reprod. Toxicol*. 6, 491-505,1992) - left testis and epididymis from all males stored frozen, homogenized and assessed for homogenization resistant sperm and sperm production rates (minimum of 200 cells if possible or 20 fields counted per sample; method of Blazak *et al*.(*Fund. Appl.Toxicol.*, 5, 1097-1103,1985). Necropsy - Pups A gross necropsy was performed on all F_1 and F_2 weanlings not selected for the F_1 and F_2 generations on postnatal day 21. Brain, spleen, pituitary, thymus, thyroid, uterus and testes weights were recorded for three randomly selected F_1 pups/sex/litter and from all F_2 weanlings not selected for neuropathology evaluation). 15 tissues and all gross lesions were collected and retained as per test guidelines. Histopathology Microscopic evaluation of the following tissues was performed on F_0 and F_1 animals (10 per sex per dose group) from the control and 750 ppm groups and any parental animals that were found dead or were euthanized *in extremis*: adrenal glands, brain, cervix, coagulating gland, epididymides (right), kidneys, lungs, nasal passages, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testis (right), thyroid, uterus and vagina and all gross lesions (from all dose groups). Blood Residue Analyses On lactation day 4, blood samples (approx. 0.5 mL) were collected approx. one hour following the third gavage dose from 4 F_1 dams/group via a tail vein. In addition, blood samples (approx. 0.5

	mL) were collected on day 22 *post partum* approx. one hour following completion of the 6 hour inhalation exposure from the same dams that were blend on lactation day 4 via a tail vein. The blood samples were collected into glass vials containing sodium heparin, sealed and stored at approx. -70ºC until they were shipped on dry ice to the University of Montreal for analysis. On postnatal day 4, blood samples were collected from culled pups from the litters of the F_1 dams that had blood collected for analyses. The blood samples were obtained by decapitation of the pups approx. 1 hour following the third gavage dose of their mothers. Blood was pooled from within each litter to obtain approx. 0.5 mL. In addition, blood samples (approx. 0.5 mL) were collected on postnatal day 22 approximately 1 hour following completion of a 6 hour inhalation exposure from the vena cava (following euthanasia by carbon dioxide inhalation) of 1 weanling/sex/litter from the same litters in the ethylbenzene exposed groups that were used in the postnatal day 4 collections. These weanlings received a single 6 hour exposure of ethylbenzene at the same concentrations as their mothers. The pup blood samples were collected and processed in the same way as the blood samples from the dam.
Results:	Parental Toxicity NOAEL – 500 ppm or 500 ppm/342 mg/kg bwt/day, NOEL – 100 ppm or 100 ppm/90 mg/kg bwt/day Reproductive Toxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt/day Developmental Toxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt/day
Remarks:	<u>Chamber Concentrations</u> Mean measured inhalation exposure concentrations were 0, 25, 100 and 501 ppm for the F_0 generation and 0, 25, 101 and 500 ppm for the F_1 generation. <u>Analysis of Dosing Solutions</u> Results of periodic concentration analyses returned the following mean concentration ranges: -----mg/mL----- <u>Target</u> <u>Actual F_0</u> <u>F_1</u> 8.67 8.27 – 8.97 7.39 – 8.94 30 30.9 – 34.5 26.3 – 32.2 114 123 – 124 98.9 - 120 *All results are presented by target inhalation or dosage level.* <u>Survival and Clinical Signs</u> There were no test article-related clinical observations or deaths. <u>Body Weight and Feed Intake</u> Body weight effects present at 500 ppm during the pre-mating period included: ➢ Reduced (16% lower) F_0 male body weight gain study week 2 to 3 ($p<0.01$) and reduced (9% lower) cumulative body weight gain study weeks 0 to 6 ($p<0.05$)

	➢ Reduced (50% lower) F_0 female body weight gain study week 1 to 2 ($p<0.05$) and reduced (6% lower) body weight study week 2 ➢ Reduced (8% lower) F_1 male body weight gain study days 28 to 35 ($p<0.05$) There were no other test-article related effects on body weight parameters and there were no effects on feed consumption parameters in the pre-mating period, the gestation period, or the lactation period. <u>Reproductive Performance</u> Ethylbenzene did not adversely affect reproductive performance in either sex from F_0 or F_1 generations. (by dose group: 0, 25, 100, 500 ppm) Mating index (%) - F_0 males: 90, 100, 97, 100 - F_0 females: 97, 100, 100, 100 - F_1 males: 88, 100, 88, 100 - F_1 females: 100, 100, 96, 100 Fertility index (%) - F_0 males: 80, 87, 77, 97 - F_0 females: 87, 87, 80, 97 - F_1 males: 84, 96, 96, 100 - F_1 females: 92, 96, 76, 96 Mean pre-coital interval (days) - F_0: 3.9, 2.9, 3.3, 2.8 - F_1: 4.2, 3.3, 4.9, 2.3 Estrous cycle length (days) - F_0: 4.4, 4.2, 4.4, 4.0* - F_1: 5.1, 5.4, 5.4, 4.4 (*statistically significantly ($p<0.01$) lower than control, however all females in this group were cycling normally, there was no impairment of mating or fertility and this strain of rat normally exhibits 4 day estrous cycles; hence the slight decrease was not considered ethylbenzene-related) <u>Gestation Length</u> The mean length of gestation was unaffected by treatment in both generations. (by dose group: 0, 25, 100, 500 ppm)(days) F_0: 22.0, 22.1, 22.0, 22.0 F_1: 22.2, 21.9, 21.9, 22.1 <u>Spermatogenic Evaluations</u> Mean testicular and epididymal sperm numbers, sperm production rate, sperm motility and morphology were comparable between control and treated males from both F_0 and F_1 generations, with no statistically significant differences present. <u>Ovarian primordial Follicle Counts</u> The mean numbers of primordial follicles for examined animals were unaffected by test article exposure. In the F_1 females, the mean numbers of primordial follicles were 129.6 and 124.8 for females in the control and 500 ppm/342 mg/kg bwt/day group, respectively.

	Necropsy Observations At the scheduled necropsies, no macroscopic or microscopic findings related to test article exposure were observed at any exposure concentration. The mean number of implantation sites did not differ between the groups: (by dose group: 0, 25, 100, 500 ppm) - F_0: 15.1, 15.0, 14.7, 15.0 - F_1: 15.6, 16.1, 16.0, 15.1 Organ Weights - F_0 -There were statistically significant ($p<0.01$) increases in absolute and relative (to final body weight) liver and kidney weights in the 500 ppm F0 males compared to the control group. The relative liver and kidney weights in the 500 ppm group were increased 13.3 and 12.5 %, respectively. In addition, there were statistically significant ($p<0.05$ or $p<0.01$) differences in absolute and/or relative (to final body weight) thyroid, lung and prostate (500 ppm group only) weights in the 100 and 500 ppm males compared to the control. The differences in prostate and lung weights were no longer significant when corrected for body weight, and, therefore, were unlikely related to exposure. In addition, the increase in lung weights in control animals may have been associated with the inflammation noted histologically. Similar changes in the weights of these organs were not seen in the F1 generation males. Absolute and relative liver weights were slightly but significantly ($p<0.01$) increased (6.6 and 7.2%, respectively) in the 500 ppm group F0 females compared to the control groups. - F_1 – There were statistically significantly ($p<0.05$ or $p<0.01$) increases in relative (to final body weight) liver and kidney weights (8.1% and 9.8%, respectively) in the 500 ppm male F_1 group when compared to the control group. Although these increases occurred in the absence of microscopic changes, similar increases in liver and kidney weights in the F_0 males at 500 ppm, hence these increases were attributed to ethylbenzene exposure. Relative liver weights were slightly increased (5.6%) in 500 ppm F_1 females when compared to controls. Although this increase occurred in the absence of macroscopic and microscopic changes, a similar increase in liver weight was observed in the F_0 females in this exposure group. Therefore, this increase was attributed to ethylbenzene exposure. Litter Data No statistically significant differences were present in litter parameters. By dose group: 0/0, 25/26, 100/90, 500/342 ppm/mg/kg bwt/day Live litter size: - F_1: 14.1, 12.8, 14.0, 14.2 - F_2: 14.7, 13.9, 14.9, 13.5

	No. live pups: - F_1: 14.3, 13.8, 14.1, 14.4 - F_2: 14.9, 14.8, 15.1, 14.0 Males/litter (%): - F_1: 48.7, 49.9, 44.9, 46.1. - F_2: 53.1, 50.5, 55.2, 47.5 <u>Pup Survival</u> Pup survival (% alive) was unaffected by treatment: By dose group: 0/0, 25/26, 100/90, 500/342 ppm/mg/kg bwt/day From birth to postnatal day 4 (pre-selection): (%) - F_1: 96.9, 93.0, 97.8, 97.2 - F_2: 97.8, 92.6, 97.0, 94.0 From postnatal day 4 (post-selection) to postnatal day 21: (%) - F_1: 95.4, 99.6, 99.5, 98.6 - F_2: 99.5, 100.0, 99.4, 100 <u>Pup body weight:</u> Mean F_1 and F_2 male and female pup body weights were unaffected by parental exposure in the control and the ethylbenzene exposure groups. Differences from control group were slight, not statistically significant and/or did not occur in an exposure related manner. <u>Pup Necropsy Findings</u> No treatment-related changes were apparent in either F_1 or F_2 pups. <u>Pre-Weaning Developmental Landmarks</u> There were no treatment-related changes to pre-weaning developmental landmarks. Mean days of acquisition By dose group: 0/0, 25/26, 100/90, 500/342 ppm/mg/kg bwt/day) Pinnal Detachment: (days) F_1 males: 4,3, 4,2, 4,3, 4,2 F_1 females: 4.2, 4.2, 4.3, 4.1 F_2 males: 4.0, 4.0, 4.0, 4.1 F_2 females: 4.0, 4.0, 4.1, 4.1 Hair Growth: (days) F_1 males: 15.0, 15.0, 15.4, 15.3 F_1 females: 15.2, 15.1, 15.4, 15.4 F_2 males: 14.2, 15.8*, 16.2*, 15.3* F_2 females: 14.4, 15.8*, 16.1*, 15.6* (statistically significantly higher ($p<0.05$ or $p<0.01$) than the control group; however, due to the lack of a clear dose-response relationship, the noticeably early appearance of hair in the concurrent control, and the absence of effects in other developmental parameters the apparent increase in all exposure groups was not attributed to parental ethylbenzene exposure. Incisor Eruption: (days) F_1 males: 9.8, 10.2, 10.5, 10.3 F_1 females: 9.9, 10.1, 10.3, 10.3

	F_2 males: 10.4, 10.6, 10.4, 10.2 F_2 females: 10.2, 10.5, 10.4, 10.2 Eye Opening: (days) F_1 males: 17.8, 18.1, 18.4, 18.1 F_1 females: 17.8, 18.1, 18.2, 18.2 F_2 males: 16.8, 17.7, 18.1, 17.2 F_2 females: 16.8, 17.7, 18.0, 17.4 <u>Post-Weaning Developmental Landmarks</u> There were no treatment-related changes to post-weaning developmental landmarks. Mean days of acquisition By dose group: 0/0, 25/26, 100/90, 500/342 ppm/mg/kg bwt/day) Balanopreputial Separation: (days) F_1 males: 43.5, 42.6, 44.3, 44.7* (*statistically significant higher ($p<0.05$) however the value was equivalent to the mean lab historical control value (44.8 days) and was not considered treatment related) F_2 males: 45.3, 45.4, 45.7, 45.5 Vaginal Patency: (days) F_1 females: 34.7, 33.3*, 33.6*, 33.9* (statistically significantly lower ($p<0.05$ or $p<0.01$) in all exposure groups compared to concurrent control, however, most likely due to a slightly elevated concurrent control mean value when compared to the mean value of the lab historical control (33.4) and hence treatment group changes not considered treatment related) F_2 females: 34.5, 36.1, 33.4, 34.0 <u>Blood Levels</u> Mean Blood Levels (mg/L) Lactation Day 4/Postnatal Day 4 from Oral Gavage <u>Group Dam Pups</u> 0 mg/kg bwt/day --- --- 26 mg/kg bwt/day 0.49 Not Detected* 90 mg/kg bwt/day 3.5 Not Detected 342 mg/kg bwt/day 18.3 Not Detected * Detection Limit = 0.006 mg/L Mean Blood levels (mg/L) Lactation Day 22/Postnatal Day 22 from Inhalation Exposure <u>Group Dam Pups</u> 0 ppm --- --- 25 ppm 0.11 0.023 100 ppm 0.56 0.281 500 ppm 11.0 12.06
Conclusion:	No parental, neonatal, or reproductive toxicity was observed following inhalation exposure of rats to up to 500 ppm or 500 ppm/342 mg/kg bwt/day ethylbenzene over two generations.

Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Stump, D.G. (2004). An inhalation two-generation reproductive toxicity study of ethylbenzene in rats including developmental neurotoxicity assessment of the F_2 generation. Study Number – WIL-186030, WIL Research Laboratories, Inc., Ashland, OH. Sponsored by the Ethylbenzene Panel, American Chemistry Council, Arlington, VA. Faber, W.D., Roberts, L.S.G., Stump, D.G., Tardif, R., Krishnan, K., Tort, M., Dimond, S., Dutton, D., Moran, E. and Lawrence, W. (2006a). Two generation reproduction study of ethylbenzene by inhalation in Crl-CD rats. *Birth Defects Research (Part B)*. 77:10-21.

[RS - 23]

Prenatal Developmental Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	"Pure Grade" (> 99%)
Method:	NIOSH Study, Comparable to Guideline Study
Type:	Developmental Toxicity Study and Screening Reproductive Toxicity Study
GLP:	Not specified
Year:	1981
Species/Strain:	Rat / Wistar
Sex:	Female
#/dose:	30
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	7 hours/day, 3 weeks before fertilization and from days 1 – 19 of gestation. Sacrifice on day 21 of gestation
Doses/Concentrations:	100, 1000 ppm – Vapor The 1000 ppm (4.34 mg/L) concentration exceeded the EPA developmental toxicity guidelines limit dose of 2 mg/L
Control Group:	Yes
Statistical Methods:	The litter was considered as the basic experimental unit for statistical analyses of the fetal data. Analysis of variance (ANOVA) was the test of choice for continuous variables when several means were compared. If results of ANOVA showed a significant treatment effect, then Duncan's multiple range test was used to make multiple comparisons among group means to investigate the possibility of a dose-response relationship. Comparison of binary response variables among groups were done by chi-square tests for independence or Fisher's Exact probability test.
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Wistar (Hilltop Lab Animals, Inc., Scottdale, PA) - Age on receipt: approximately 5 weeks (females) and 7 weeks

	(males) - Body weight on receipt: 150 g (females); male weight not specified - Acclimation period: 3-4 weeks - Housing: individually in stainless steel wire cages - Diet: Wayne Lab-Blox, *ad libitum* except during exposure - Water: *ad libitum* except during exposure Chambers Exposures were conducted in 2.3 m^3 stainless steel chambers. Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph equipped with a flame ionization detector. Each study chamber atmosphere was analyzed approximately twice per hour during the 7 hour exposure. Exposure Regimen: Pregestational / Gestational Group — Dose before Fertilization (ppm) — Dose After Fertilization (ppm) Control: 0 / 0 I: 0 / 100 II: 0 / 1000 III: 100 / 0 IV: 100 / 100 V: 1000 / 0 VI: 1000 / 1000 Assessments Clinical observations, body weights, and feed consumption measurements were collected throughout the experiment. On day 21 of gestation, the females were sacrificed by decapitation and the uterine contents were examined. The internal organs of the dams were examined grossly and liver, spleen, lung, and kidney weights were collected. The ovaries, uterus, liver, lungs (with trachea) and kidneys were processed and examined for histopathological changes. Implantations, dead and resorbed fetuses, and corpora lutea counts were recorded. Pre- and post-implantation losses and number and percent of liver offspring were recorded. Placental, but not gravid uterine weights were collected and recorded. Fetal data collected included sex ratio, body weights (sexes combined), crown-rump lengths, and external observations. The heads of approximately one-half of the fetuses in each litter were removed, preserved in Bouin's fixative, sectioned, and inspected. The decapitated fetuses were subjected to a fresh visceral examination (Staples technique). The fetuses not decapitated were eviscerated and processed for skeletal examination.
Results:	Maternal Toxicity NOAEL – 1000 ppm, NOEL – 100 ppm Developmental Toxicity NOAEL – 100 ppm
Remarks:	Chamber Concentrations Actual mean ethylbenzene exposure concentrations achieved in the chambers were 97 and 959 ppm for the pregestational exposures and 96 and 985 ppm for the gestational exposures. *All results are presented by target inhalation level.*

	Maternal Effects One rat in the 1000 ppm pregestational exposure group died of unknown causes on the 8th day of pregestational exposure, however there was no evidence of toxicity observed in the remaining rats or other groups. Body weights were generally unaffected during the pregestational and gestational periods. Increased absolute and relative weights of liver (~22%), kidneys (~10%), and spleen (~10%)(groups significantly different by ANOVA, Duncan's) were present in 1000 ppm dams only (Groups II and VI) with no accompanying histopathological changes. Reproductive Effects There was no treatment-related effect on fertility or on any other measure of reproductive status. A higher percentage of ethylbenzene exposed females mated (were sperm positive) than controls (67, 78, and 74% for 0, 100, and 1000 ppm, respectively) and a slightly smaller percentage of ethylbenzene exposed females that mated were pregnant at gestation day 21 (89, 77 and 77% for 0, 100, and 1000 ppm, respectively)($p \leq 0.05$). When expressed on the basis of total females per group, 56, 60, and 57% of females exposed to 0, 100, 1000 ppm were pregnant at gestation day 21. Thus exposure of female rats to ethylbenzene at 100 or 1000 ppm for three weeks did not decrease fertility. Developmental Effects There were no significant increases in major malformations or minor anomalies in any of the ethylbenzene exposed groups. With the exception of supernumerary ribs, there were no significant effects of exposure on the incidence of common variations. The incidence of extra ribs was statistically increased in fetuses in both of the rat groups exposed to 1000 ppm during gestation (Groups II and VI) (3, 27.6, and 22.6% in control, Group II and Group VI, respectively)($p \leq 0.05$) and to 100 ppm during gestation (Group 1)(3 and 18.2% for control and Group 1, respectively) ($p \leq 0.05$), while rudimentary rib incidence was elevated significantly only in fetuses in the rat group exposed to 1000 ppm during gestation (Group II)(39.4 and 69% for control and Group II, respectively) ($p \leq 0.05$). When the incidences of these two skeletal variants are combined (supernumerary ribs) that of the 1000 ppm during gestation only (Group II)(36.4 and 69% for control and Group II, respectively) ($p \leq 0.05$) but not that for the 1000 ppm pre-gestation (36.4 and 37.9 for control and Group V, respectively) and gestation (Group VI)(36.4 and 51.6 for control and Group VI, respectively) was significant. Interpretation of these results is difficult in the absence of a clear dose-response relationship. When only gestational exposure is considered for comparative purposes, then only the 1000 ppm exposed groups had an increased incidence of supernumerary ribs on the basis of percent of litters affected (69.0, versus 51.6% for 1000 ppm during gestation only and 1000 ppm pre-gestation and gestation, respectively). The range for all of the air- and 100 ppm exposed rats was 36.4-48.5%. Thus, there appears to be an increase in this class of abnormalities as a result of exposure to ethylbenzene at 1000 ppm. The skeletal variants in this study are considered marginally adverse.
Conclusion:	Ethylbenzene produced a mild increase in fetal effects (increased incidence in skeletal variations) in concert with mild maternal

	increases in organ weights at 1000 ppm.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. The study design was similar to current guidelines and some parameters exceeded current guidelines (e.g. exposure initiated on the day after impregnation, histopathology was conducted on certain maternal organs). Although only 2 exposure concentrations were assessed, the high concentration exceeded EPA's developmental toxicity guideline limit dose. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Andrew, F.D., Bushbom, R.L., Cannon, W.C., Miller, R.A., Montgomery, L.F., Phelp, D.W., and Sikov, M.R. (1981). Teratologic assessment of ethylbenzene and 1-ethoxyethanol. Battelle Pacific Northwest Laboratories. Prepared for the National Institute for Occupational Safety and Health, Cincinnati, OH. NIOSH Contract #210-79-0037. Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P. and Niemeier, R.W. (1981). Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work. Environ. Health*. 7 (Suppl. 4):66-75.

[RS - 24]

Prenatal Developmental Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	"Pure Grade" (> 99%)
Method:	NIOSH Study, Comparable to Guideline Study
Type:	Developmental Toxicity Study
GLP:	Not specified
Year:	1981
Species/Strain:	Rabbit / New Zealand White
Sex:	Female
#/dose:	21 – 24
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	7 hours/day, Days 1 – 24 of gestation. Sacrifice on day 30 of gestation
Doses/Concentrations:	100, 1000 ppm – Vapor The 1000 ppm (4.34 mg/L) concentration exceeded the EPA developmental toxicity guidelines limit dose of 2 mg/L
Control Group:	Yes
Statistical Methods:	The litter was considered as the basic experimental unit for statistical analyses of the fetal data. Analysis of variance (ANOVA) was the test of choice for continuous variables when several means were compared. If results of ANOVA showed a significant treatment effect, then Duncan's multiple range test was used to make multiple comparisons among group means to investigate the possibility of a dose-response relationship. Comparison of binary response variables among groups were done by chi-square tests for independence or Fisher's Exact probability test.
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rabbit, New Zealand White rabbit (White's, Kootenai, ID)(males were proven breeders of the same strain retained from a previous study, original source not specified) - Age on receipt: 4.5-6 months (females) - Acclimation period: approximately 28 days

	- Housing: individually housed in stainless steel wire cages - Diet: Wayne Rabbit Diet, *ad libitum* except during exposure - Water: *ad libitum* except during exposure Chambers Exposures were conducted in 2.3 m^3 stainless steel chambers. Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph equipped with a flame ionization detector. Each study chamber atmosphere was analyzed approximately twice per hour during the 7 hour exposure. Assessments Clinical observations, body weights, and feed consumption measurements were collected throughout the experiment. On day 30 of gestation, the females were sacrificed by decapitation and the uterine contents were examined. The internal organs of the does were examined grossly and liver, spleen, lung, and kidney weights were collected. The ovaries, uterus, liver, lungs (with trachea) and kidneys were processed and examined for histopathological changes. Implantations, dead and resorbed fetuses, and corpora lutea counts were recorded. Pre- and post-implantation losses and number and percent of liver offspring were recorded. Placental, but not gravid uterine weights were collected and recorded. Fetal data collected included sex ratio, body weights (sexes combined), crown-rump lengths, and external observations. All fetuses were subjected to a fresh visceral examination (Staples technique) and a skeletal examination. The heads of approximately one-half of the fetuses in each litter were removed, preserved in Bouin's fixative, sectioned, and inspected.
Results:	Maternal Toxicity NOAEL – 1000 ppm, NOEL – 100 ppm Developmental Toxicity NOAEL – 1000 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 99 and 962 ppm ethylbenzene. *All results are presented by target inhalation level.* Maternal Effects There were no treatment-related effects on maternal survival or body weight. There was no evidence of histologic damage in any of the does' organs. Relative liver weights were increased (16%) in 1000 ppm does (significantly different from controls by ANOVA, Duncan's) but absent any accompanying histopathological changes, this finding was not considered biologically relevant. Developmental Effects There were no treatment-related developmental toxic effects. There was a slight, but statistically significant decrease in the number of live fetuses/litter in the 1000 ppm group (8 ± 3 in the control group versus 7 ± 3 in the 1000 ppm group; groups were significantly different by ANOVA, Duncan's). The finding was considered equivocal due to no corresponding increases in other parameters (implantations, resorptions, dead fetuses, etc.). There were no significant changes in

	the incidence of variations or malformations in the rabbit pups.
Conclusion:	Ethylbenzene did not elicit maternal or developmental toxicity in rabbits at 1000 ppm.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. The study design was similar to current guidelines and some parameters exceeded current guidelines (e.g. exposure initiated on the day after impregnation, histopathology was conducted on certain maternal organs. Although only 2 exposure concentrations were assessed, the high concentration exceeded EPA's developmental toxicity guideline limit dose. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Andrew, F.D., Bushbom, R.L., Cannon, W.C., Miller, R.A., Montgomery, L.F., Phelp, D.W. and Sikov, M.R. (1981). Teratologic assessment of ethylbenzene and 1-ethoxyethanol. Battelle Pacific Northwest Laboratories. Prepared for the National Institute for Occupational Safety and Health, Cincinnati, OH. NIOSH Contract #210-79-0037. Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P. and Niemeier, R.W. (1981). Testing of selected workplace chemicals for teratogenic potential. *Scand. J. Work. Environ. Health*. 7 (Suppl. 4):66-75.

[RS - 25]

Prenatal Developmental Toxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	≥ 99.5 %
Method:	Designed in Conformance with EPA (1998) and OECD (2001) Guidelines
Type:	Developmental Toxicity Study
GLP:	Not specified but presumably Yes
Year:	2003
Species/Strain:	Rat / Sprague-Dawley
Sex:	Female
#/dose:	20 – 26
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, during days 6 – 20 of gestation Sacrifice on day 21 of gestation
Doses/Concentrations:	100, 500, 1000, 2000 ppm – Vapor
Control Group:	Yes
Statistical Methods:	The litter was considered as the basic experimental unit for statistical analyses of the fetal data. The number of corpora lutea, implantation sites and live fetuses, maternal feed consumption, and various body weights were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's test if differences were found. The percentage of non-live implants, resorptions, and males, and the proportions of fetuses with alterations in each litter were evaluated by the Kruskal-Wallis test followed by the Mann-Whitney test where appropriate. Rates of pregnancy and percentages of litters with any malformations or external, visceral or skeletal variations were analyzed by using Fisher's test. Where applicable, least-squares analysis was carried out. The reported level of statistical significance was $P < 0.05$.
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Sprague-Dawley (IFFA CREDO Breeding Laboratories, Saint-Germain-sur-l'Arbresle, France) - Sex: females - Age on receipt: not specified

	- Acclimation period: 2 weeks - Housing: individually housed in polycarbonate cages with stainless steel wire lids - Diet: food pellets (UAR Alimentation Villemoisson, France), *ad libitum* except during exposure - Water: filtered tap water, *ad libitum* except during exposure Environment: controlled to 21±2 ºC, 50 ± 5% relative humidity, 12 hour light/dark cycle - Age at first treatment: not specified - Body weight at first treatment: 180-200 g <u>Chambers</u> Exposures were conducted in 200-L glass/stainless steel inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were monitored by a sampling system coupled to a gas chromatograph equipped with a flame ionization detector. <u>Assessments</u> Clinical observations, body weights, and feed consumption measurements were collected throughout the experiment. On day 21 of gestation, the females were sacrificed with an interpulmonary injection of T-61. The uterus was removed and weighed. The number of corpora lutea, implantation sites, resorptions, dead and live fetuses were recorded. Live fetuses were weighed, sexed, and examined for external anomalies including those of the oral cavity. Half of the live fetuses were preserved in Bouin's solution and examined for internal soft tissue changes by the method of Wilson. The other half were fixed in 70% ethanol, eviscerated, and then processed for skeletal staining with Alizarin Red S for subsequent skeletal examination (Staples technique).
Results:	Maternal Toxicity NOAEL – 500 ppm Developmental Toxicity NOAEL – 500 ppm
Remarks:	<u>Chamber Concentrations</u> Actual mean ethylbenzene exposure concentrations achieved in the chambers were 99, 500, 1001 and 1998. *All results are presented by target inhalation level.* <u>Maternal Effects</u> Ethylbenzene exposure did not cause maternal death. Clinical signs of toxicity (ataxia, decreased motor activity) were seen at 2000 ppm. Maternal body weight was significantly reduced (7%) on gestation day 21 at 1000 ppm ($p<0.05$) and on gestation day 13 (8.5%) and 21 (18%) at 2000 ppm ($p<0.01$). Dams exposed to 1000 or 2000 ppm showed significant decreases in maternal weight gain and feed consumption throughout exposure and in corrected weight gain ($p<0.05$ or $p<0.01$). <u>Developmental Effects</u> The number of implantations was comparable among groups. Although the difference was not statistically significant, the incidence of non-live implants (mean of 21.4 versus 5.2 for controls) and resorptions (mean of 20.2 versus 5.2) was higher at 2000 ppm than in

	the control group. This was likely due to the 100% post-implantation loss seen in 3 of the 21 pregnant females exposed to 2000 ppm (0 in other groups). Ethylbenzene produced a concentration-related reduction in fetal weights that achieved statistical significance at 1000 ppm (5.31 and 4.70 g in 1000 and 2000 ppm groups, respectively versus 5.70 g in controls)($p<0.01$). These decreases amounted to 7 and 18% of the control values at 1000 and 2000 ppm, respectively. No evidence of teratogenic effects were found at any exposure level. Visceral malformations occurred in one or few fetuses from the 100, 1000 and 2000 ppm exposure groups, without a clear concentration-dependent relationship. No significant differences were observed between the control and treated groups in the incidences of either individual or total external or visceral variations, or individual skeletal variations. There was an increased number of fetuses with skeletal or any variations at 1000 and 2000 ppm. The mean percentage of fetuses per litter with skeletal or any variations was also significantly increased at 2000 ppm (35.6% versus 16.1% in controls; $p<0.01$).
Conclusion:	Ethylbenzene produced a mild increase in fetal effects, indicated by reductions in fetal body weight and a higher incidence of skeletal variations, in concert with mild maternal effects (clinical signs and body weight decreases) in rats at $\geq$ 1000 ppm.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Saillenfait, A.M., Gallissot, F., Morel, G. and Bonnet, P. (2003). Developmental toxicities of ethylbenzene, *ortho-, meta-, para*-xylene and technical xylene in rats following inhalation exposure. *Food and Chemical Toxicology*. 41:415-429.

[RS - 26]

Immunotoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.9%
Method:	EPA Health Effects Testing Guidelines OPPTS 870.7800 - Immunotoxicity
Type:	Inhalation Splenic Antibody Formation Study in Rats
GLP:	Yes
Year:	2004
Species/Strain:	Crl:CD® (Sprague-Dawley) IGS BR
Sex:	Females
#/dose:	10
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	Daily 6 hour exposures for 28 consecutive days
Doses/Concentrations:	25, 100, 500 ppm - Vapor
Control Group:	Negative Control – Air only Positive Control – Cyclophosphamide (CP, intraperitoneal injection, 50 mg/kg bwt/day at 10 mg/mL, injections given daily for 4 consecutive days through the day prior to the scheduled necropsy)
Statistical Methods:	Parametric one-way analysis of variance (ANOVA) – body weight, body weight changes, feed consumption, hematology parameters and organ weights; if the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test was used. Splenic antibody-forming cell (AFC) assay data was first tested for homogeneity of variances using the Bartlett's Chi-Square test. Homogeneous data were evaluated using ANOVA. When significant differences occurred, the treatment groups were compared to the vehicle control group using Dunnett's test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, the treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test when appropriate. The Jonckheere's test was used to test for dose-related trends across the vehicle and

	treatment groups. The positive control data were evaluated using the Student's *t*-test and compared to the vehicle control. The criteria for accepting the results of the positive control group in the assay were statistically significant ($p \leq 0.05$) decreases in the response compared to the response of the vehicle control group.
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Crl:CD (SD)IGS BR (Charles River Laboratories, Raleigh, NC) - Sex: Females. Female rats were used for the study based on essentially similar toxicity profile in males and female rats in subchronic studies and preference for the more docile female rat for the intravenous tail injection procedure. - Age: 38 days on receipt - Acclimation period: 7 days - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum* except during exposure - Water: reverse osmosis-treated tap water, *ad libitum* - Environment: controlled to 71 ± 5 ºF, 50 ± 20% relative humidity, 12 hour light/dark cycle, 10 air changes/hour - Age at first treatment: approximately 7 weeks - Body weight at first treatment: 142 – 200 g <u>Chambers and Treatments</u> Rats were exposed in 2 m^3 stainless steel and glass whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured at least 10 times per exposure day by an automatic sampling system coupled to a gas chromatograph. Target test article concentrations were 25, 100, and 500 ppm. The highest exposure level of 500 ppm (2.17 mg/L) exceeded the recommendation given in OPPTS 870.7800 for an upper limit dose of 2 mg/L. All animals received a single intravenous immunization injection via a lateral tail vein of sheep red blood cells (sRBCs) approximately 4 days prior to the scheduled necropsy. <u>Assessments</u> All animals were observed twice daily for moribundity and mortality and weekly detailed physical examination data were collected. Animals were observed daily for clinical changes prior to exposure and within 1 hour after completion of each exposure period. Body weights and feed consumption were recorded twice weekly until study termination. Blood was collected for hematology evaluations from all animals at the time of the scheduled necropsy (study week 4). In addition, serum was obtained and stored frozen for potential future IgM antibody assay. Complete necropsies were conducted on all animals and selected organs were weighed. Splenic tissues were collected from all animals at the scheduled necropsy. The splenic samples were

	randomized for AFC analysis so that the analyst was unaware of the treatment group of each sample examined. The AFC response was evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen).
Results:	NOAEL – 500 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 25.0, 100.9, and 500.4 ppm ethylbenzene. *All results are presented by target inhalation level.* Survival, Clinical Signs, Body Weights There were no treatment related effects on survival, clinical signs, body weight, or feed consumption. Clinical Pathology There were no treatment related effects on hematology parameters. Pathology Liver and kidney weights relative to final body weights were increased (13% for both) in the 500 ppm group. The positive control article, CP, performed as expected, exhibiting a decrease in spleen and thymus weights. AFC There were no treatment related effects of ethylbenzene on IgM Antibody Forming Cell Response. The positive control article, CP, performed as expected, exhibiting a decrease in spleen cell numbers and a decrease in IgM Antibody Forming Cell Response. Summary of Mean Results of AFC Assay Ethylbenzene (ppm) / CP (mg/kg bwt) 0 / 25 / 100 / 500 / 50 Spleen wt (mg): 610 / 548 / 539 / 562 / 271* % body wt: 0.252 / 0.244 / 0.232 / 0.253 / 0.132* spleen Cells (x10^7): 78.74 / 65.96 / 63.76 / 67.90 / 9.67* IgM AFC/10^6 spleen cells: 1473 / 1546 / 1718 / 2145 / 40* IgM AFC/Spleen (10^3): 1134 / 1027 / 1168 / 1462 / 4* Thymus wt (mg): 486 / 424 / 455 / 532 / 107 % body wt: 0.198 / 0.191 / 0.197 / 0.194 / 0.053* * Statistically significant at $p < 0.01$ using Dunnett's Test
Conclusion:	Ethylbenzene at up to 500 ppm vapor concentration did not adversely affect the functional ability of the humoral immune component of the rat immune system as measured by splenic IgM antibody forming cell (AFC) response to the T-dependent antigen, sheep erythrocytes.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.

Reference:	Stump, D.G. (2004). A 28-day inhalation splenic antibody formation study of ethylbenzene in rats. Study Number: WIL-186029. WIL Research Laboratories, Inc., Ashland, OH, Sponsored by the Ethylbenzene Panel, American Chemistry Council, Arlington,VA.

[RS - 27]

Chronic Toxictiy / Carcinogenicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Ethylbenzene was used from two lots: A060989 and A051890; A060989 had an overall purity > 99% and contained 62 +/- 3.1 ppm cumene; lot A051890 also had an overall purity of > 99%; Concentration of peroxide ranged from 1.12 to 10.7 ppm
Method:	NTP Study, Comparable to Guideline Study
Type:	Combined Chronic Toxicity and Carcinogenicity Study
GLP:	Yes
Year:	1999
Species/Strain:	Rat / Fischer 344/N
Sex:	Male and Female
#/sex/dose:	50
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week for 104 weeks
Doses/Concentrations:	75, 250, 750 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	Animals and Maintenance - Species and strain: rat, Fischer 344/N (Simonsen Laboratories, Inc., Gilroy, CA) - Sex: males and females - Age at receipt: not specified - Acclimation period: 13 days - Housing: individually housed in stainless steel cages - Diet: NIH-07 open formula pelleted diet (Zeigler Brothers Inc., Gardners, PA), *ad libitum* except during exposure - Water: untreated course-filtered City of Chicago water, *ad libitum* except during exposure Environment: controlled to 21-28 ºC, 37-76% relative humidity, 12 hour light/dark cycle - Age at first treatment: approximately 6 weeks

	Chambers Exposures were conducted in stainless steel chambers. Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph equipped with a flame ionization detector. Each study chamber atmosphere was analyzed hourly during the 6 hour exposure. Assessments The animals were observed twice daily for clinical signs of toxicity. Body weights were recorded weekly for 13 weeks and monthly from week 16 to the end of the study. A complete necropsy and microscopic examination were performed on all animals. At necropsy all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and preserved in 10% neutral buffered formalin, and processed for microscopic examination. Evaluation of Results/Statistical Analyses Statistical analyses for possible dose-related effects on survival used Cox's method for testing two groups for equality and Tarone's life table test to identify dose-related trends. The incidences of neoplasms and nonneoplastic lesions were calculated as were the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. The majority of the neoplasms in these studies were considered incidental to the cause of death or not rapidly lethal. Thus the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasm were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. Neoplastic prevalence was modeled as a logistic function of chemical exposure and time. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. In addition to logistic regression other methods employed were the life table test appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test, procedures based on the overall proportion of neoplasm-bearing animals. Tests of significance included pair wise comparisons of each exposed group with controls and a test for an overall dose related trend. For the analysis of nonneoplastic lesions, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. Average severity values were analyzed for significance with the Mann-Whitney test.
Results:	Chronic Toxicity NOAEL – 250 ppm (males), < 75 ppm (females) Carcinogenicity NOAEL – 250 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 74.8, 250 and 749 ppm ethylbenzene. *All results are presented by target inhalation level.*

	Survival and Body Weights At 750 ppm, survival in males was significantly reduced (animals surviving to study termination 2/50 for 750 ppm vs. 15/50 in controls)($p<0.001$), while in females survival was increased (not significant). The survival of male rats followed a negative trend, decreasing with increasing dose. Mean body weights of 250 ppm and 750 ppm males were generally lower than those of the chamber controls (up to 5 and 15%, respectively) from week 20. Mean body weights of exposed groups of females were generally lower than those of the chamber controls during the second year of the study. No clinical findings were attributed to ethylbenzene exposure. Pathological Findings Test article-related organ pathology was present in the kidney and testes of ethylbenzene exposed rats. *Kidney* In the kidney, the standard histopathological evaluation found a significantly greater incidence in the 750 ppm male rats of renal tubule adenoma (4/50 vs. 0/50 in controls; $p\leq0.05$) and adenoma or carcinoma (combined)(7/50 vs. 0/50; $p\leq0.01$) than found in the chamber controls. The incidence of renal tumors in 750 ppm males exceeded the NTP historical control range for this tumor type. In addition, the incidence of renal tubule hyperplasia in 750 ppm males was significantly greater than that in the chamber control group (12/50 vs. 2/50 in controls; $p\leq0.01$). The findings from an extended evaluation (step section) of the kidney showed a significant increase in the incidences of renal tubule adenoma (17/50 vs. 3/50 in controls for males, $p\leq0.01$, and 7/49 vs. 0/50 in controls for females, $p\leq0.05$) and renal tubule hyperplasia in 750 ppm males and females (17/50 vs. 10/50 in controls for males, $p\leq0.05$, and 8/49 vs. 1/50 in controls for females, $p\leq0.05$); the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 750 ppm males (18/50 vs. 3/50 in controls; $p\leq0.01$). The severity of nephropathy was significantly increased relative to the chamber controls in 750 ppm male (3.5 vs. 2.3 in controls; $p\leq0.01$) and all exposed female rats (2.3, 1.7, 1.6 and 1.3 for 750, 250, 75 ppm and control groups; $p\leq0.05$ or $p\leq0.01$). The enhanced nephropathy was more severe in males than in females. *Testis* In the testis, the incidence of interstitial cell adenoma in 750 ppm males was significantly greater than in the chamber control group (44/50 vs. 36/50 in controls) and slightly exceeded the NTP historical control range for inhalation studies. The incidence of bilateral testicular adenoma was also significantly increased at 750 ppm (40/50 vs. 27/50 in controls), whereas the incidence of interstitial cell hyperplasia was significantly decreased at this concentration level (8/50 vs. 14/50 in controls; $p\leq0.05$). Although testicular adenoma will develop in nearly all aged Fischer rats, ethylbenzene appeared to enhance its development since 92% (22 of 24 rats) of the 750 ppm rats that died between day 400 and day 600 had testicular adenoma, whereas only 33% (3 of 9 rats) of the control that died early had testicular adenoma.

	Other Organs 750 ppm males exhibited increase lesion incidences of edema (1/50, 0/50, 0/50, 6/50 for chamber control, 75, 250, and 750 ppm), congestion (1/50, 2/50, 0/50, 6/50 for chamber control, 75, 250, and 750 ppm), and hemorrhage (0/50, 2/50, 1/50, 8/50 for chamber control, 75, 250, and 750 ppm) in the lungs as well as hemorrhage in mesenteric (3/49, 5/50, 4/50, 8/50 for chamber control, 75, 250, and 750 ppm) and renal (0/9, 0/8, 1/9, 8/14 for chamber control, 75, 250, and 750 ppm) lymph nodes were slightly increased. These circulatory lesions were considered to be agonal changes in moribund animals and not directly related to chemical toxicity. The incidences of cystic degeneration of the liver was also increased in 750 ppm males (15/50, 12/50, 19/50, 30/49 for chamber control, 75, 250, and 750 ppm); the biologic significance of this increase in the absence of other hepatotoxic changes was deemed unclear. Compared to the chamber control group, the incidences of prostate gland inflammation in all exposed groups of males were significantly increased (11/50, 29/50, 22/50, 25/50 for chamber control, 75, 250, and 750 ppm). This inflammatory change consisted of infiltration by predominately mononuclear inflammatory cells with glandular acini and interstitium, increased interstitial fibrosis, and loss of secretory material in affected areas. Relative to chamber controls, males exposed to 75 or 750 ppm exhibited increased incidences of hyperplasia of the bone marrow characterized by hypercellularity due to the increased numbers of erythroid and myeloid precursor cells (7/49, 16/49, 9/50, 19/50 for chamber control, 75, 250, and 750 ppm). The relationship of these changes to ethylbenzene exposure is uncertain due to the lack of clear concentration-dependent responses. *Cancer Conclusions* According to NTP, there was clear evidence of carcinogenicity in male rats due to increased incidences of kidney (renal tubule neoplasms) and testes tumors (testicular adenoma) and some evidence of carcinogenicity in female rats that also showed kidney tumors (renal tubule adenomas), but in a lower incidence and only detected after extended evaluation by step sections. <u>Follow-Up Information</u> The kidney slides from this study were re-examined (Hard, 2002). Kidney slides were evaluated for hyaline droplet accumulation, sustained cytotoxicity/cell regeneration, interaction with chronic progressive nephropathy (CPN), and tumors. Ethylbenzene caused an exacerbation of age-related spontaneous renal disease, CPN, in the 750 ppm animals, markedly so in the male rats, and modestly so in the females. In addition, there was a high incidence of high-dose rats that had end-stage CPN, a terminal condition where the kidneys are so morphologically altered that renal failure (as well as secondary hyperthyroidism) occurs. Although there some evidence of a dose-related increase in hyaline droplet formation in the 13-week NTP study, it was not considered to be of the magnitude indicative of an α-2u-globulin associated mechanism of renal carcinogenesis. Other pathological effects associated with α-2u-globulin were absent in the male rat kidneys from the 2-yr NTP study. The author concluded that the re-evaluation of this study provided persuasive evidence that the apparent increase in renal

	tumors was strongly associated with CPN, a spontaneous age-related disease of rodents with no identical counterpart in humans.
Conclusion:	Chronic Toxicity - A decrease in survival and body weight and an increase in kidney pathology (renal tubule hyperplasia and nephropathy) were observed in rats that inhaled ≥ 75 ppm ethylbenzene for 2 years. Cancer – At 750 ppm ethylbenzene, male rats exhibited increased incidences of kidney (renal tubule neoplasms) and testes tumors (testicular adenoma) and female rats also showed kidney tumors (renal tubule adenomas), but in a lower incidence and only detected after extended evaluation.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1999). Toxicology and carcinogenesis studies of ethyl-benzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation studies) (Tech. Rep. Ser. No. 466; NIH Publ No. 99-3956), National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park, NC. Hard, G.C. (2002). Significance of the renal effects of ethylbenzene for assessing human carcinogenic risk. *Toxicol. Sci.* 69:30-41.

[RS - 28]

Chronic Toxicity / Carcinogenicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	Ethylbenzene was used from two lots: A060989 and A051890; A060989 had an overall purity > 99% and contained 62 +/- 3.1 ppm cumene; lot A051890 also had an overall purity of > 99%; Concentration of peroxide ranged from 1.12 to 10.7 ppm
Method:	NTP Study, Comparable to Guideline Study
Type:	Combined Chronic Toxicity and Carcinogenicity Study
GLP:	Yes
Year:	1999
Species/Strain:	Mouse / B6C3F1
Sex:	Male and Female
#/sex/dose:	50
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 5 days/week for 104 weeks
Doses/Concentrations:	75, 250, 750 ppm – Vapor
Control Group:	Yes
Remarks for Test Conditions:	Animals and Maintenance - Species and strain: mouse, B6C3F1 (Simonsen Laboratories, Inc., Gilroy, CA) - Sex: males and females - Age at receipt: not specified - Acclimation period: 11 days - Housing: individually housed in stainless steel cages - Diet: NIH-07 open formula pelleted diet (Zeigler Brothers Inc., Gardners, PA), *ad libitum* except during exposure - Water: untreated course-filtered City of Chicago water, *ad libitum* except during exposure Environment: controlled to 21-27 ºC, 32-72% relative humidity, 12 hour light/dark cycle - Age at first treatment: approximately 6 weeks

	Chambers Exposures were conducted in stainless steel chambers. Concentrations of ethylbenzene in the inhalation chambers were monitored by an automatic sampling system coupled to a gas chromatograph equipped with a flame ionization detector. Each study chamber atmosphere was analyzed hourly during the 6 hour exposure. Assessments The animals were observed twice daily for clinical signs of toxicity. Body weights were recorded weekly for 13 weeks and monthly from week 16 to the end of the study. A complete necropsy and microscopic examination were performed on all animals. At necropsy all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and preserved in 10% neutral buffered formalin, and processed for microscopic examination. Evaluation of Results/Statistical Analyses Statistical analyses for possible dose-related effects on survival used Cox's method for testing two groups for equality and Tarone's life table test to identify dose-related trends. The incidences of neoplasms and nonneoplastic lesions were calculated as were the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. The majority of the neoplasms in these studies were considered incidental to the cause of death or not rapidly lethal. Thus the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasm were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. Neoplastic prevalence was modeled as a logistic function of chemical exposure and time. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. In addition to logistic regression other methods employed were the life table test appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test, procedures based on the overall proportion of neoplasm-bearing animals. Tests of significance included pair wise comparisons of each exposed group with controls and a test for an overall dose related trend. For the analysis of nonneoplastic lesions, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. Average severity values were analyzed for significance with the Mann-Whitney test.
Results:	Chronic Toxicity NOAEL – 75 ppm Cancer NOAEL – 250 ppm
Remarks:	Chamber Concentrations Actual mean exposure concentrations achieved in the chambers throughout the study were 75.2, 248 and 748 ppm ethylbenzene. *All results are presented by target inhalation level.*

	Survival and Body Weights Survival of exposed groups of male and female mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 75 ppm, were greater than those of the chamber controls from week 72 until the end of the study. Mean body weights of 750 ppm females were generally less than those of the chamber controls from week 24 through week 68 but were similar to those of the chamber control from week 72 until the end of the study. No clinical findings were attributed to ethylbenzene exposure. Pathological Findings Test article-related organ pathology was present in the lung, liver, thyroid gland and pituitary gland of ethylbenzene exposed mice. *Lung* In the lung, 750 ppm male mice, compared to the chamber control group, exhibited a significantly greater incidence of alveolar/bronchiolar adenoma (16/50 vs. 5/50 in controls; p≤0.01) and alveolar/bronchiolar adenoma or carcinoma (combined)(19/50 vs. 7/50 in controls; p≤0.01) although these incidences were within the NTP historical control range. The incidence of alveolar epithelial metaplasia in 750 ppm males was significantly greater than that in the chamber controls (6/50 vs. 0/50 in controls; p≤0.05). There was no increase in alveolar hyperplasia in males and no significant increase in the incidence of either hyperplasia or metaplasia in females. No effects were observed in either male or female mice at 250 or 75 ppm. *Liver* In the liver, the incidences of hepatocellular adenoma (16/50 vs. 6/50 in controls; p≤0.05) and hepatocellular adenoma or carcinoma (combined)(25/50 vs. 13/50 in controls; p≤0.05) were significantly greater in 750 ppm female mice than those in the chamber control group but were within the NTP historical range. There was a spectrum of nonneoplastic liver changes related to ethylbenzene exposure in female and male mice. Females (but not males) exposed to 750 ppm had an increased incidence of eosinophilic foci (22/50 vs. 5/50 in controls; p≤0.01), a lesion which is judged to be a precursor of hepatocellular adenomas. The incidence of eosinophilic foci in either males or females exposed to 250 or 75 ppm was not significantly different from the control incidences. There were, however, increased incidences (p≤0.01) of hepatocyte syncytial alteration, hypertrophy and necrosis in the liver of males exposed to 750 ppm ethylbenzene compared to controls (23/50 vs. 0/50, 17/50 vs. 1/50, 10/50 vs. 1/50, respectively) and increased syncytial alteration of hepatocytes of 250 ppm males (8/50 vs. 0/50 in controls; p≤0.01). Histological lesions consistent with *Heliobacter hepaticus* infection were not identified in livers of mice in this study. Based upon evidence from NTP studies, the presence of *Heliobacter* in the absence of histological evidence of liver disease was not considered to significantly compromise the host's response to administration of ethylbenzene. *Other Organs* Positive trends in the incidences of thyroid follicular cell hyperplasia

	occurred in both males (21/50, 21/50, 29/50, 32/50 for chamber controls 0, 75, 250, and 750 ppm) and females (18/50, 23/50, 25/50, 35/50 for chamber controls 0, 75, 250, and 750 ppm), with significant increases in incidences relative to chamber controls in 750 ppm males and females. Thyroid hyperplasia was typically a focal noncompressive proliferation with simple papillary infoldings of follicular epithelial cells. Significantly increased incidences of hyperplasia of the pituitary gland pars distalis were limited to 250 and 750 ppm females (10/48, 12/49, 23/47, 22/49 for chamber controls 0, 75, 250, and 750 ppm). This hyperplasia was seen as focal, poorly delinated, monomorphic increases in cells which had no compressive features or altered arrangement. There were no corresponding increases in the incidences of adenomas of either the thyroid or pituitary gland. *Cancer Conclusion* According to NTP there was some evidence of carcinogenicity in both sexes; for male mice due to lung tumors (alveoar/bronchiolar neoplasms) and for female mice due to liver tumors (hepatocellular neoplasms). <u>Follow-Up Information</u> The lung and liver sections of mice from the National Toxicology Program (NTP) two-year bioassay were re-evaluated by Brown (2000). This re-evaluation revealed an increased incidence of male and female mice of the 750 ppm exposure group with decreased eosinophilia of the terminal bronchiolar epithelium. Also, a dose-related increased incidence in multifocal hyperplasia of the bronchiolar epithelium with extension to the peribronchiolar alveolar epithelium was observed in all male treated groups and mid- and high-exposure females. The author noted that the necrotic hepatocytes in the high-dose males were usually that of a coagulation-type necrosis of single or small groups of cells, usually the enlarged, hypertrophied centrilobular hepatocytes. The morphology of this necrosis was histomorphologically different from "apoptosis." Also, the syncytial cells were not the predominant cell type with necrosis.
Conclusion:	Chronic Toxicity - Liver, lung, thyroid and pituitary pathology was observed in mice that inhaled ≥ 250 ppm ethylbenzene for 2 years. Cancer - At 750 ppm ethylbenzene, male mice exhibited lung tumors (alveoar/bronchiolar neoplasms) and female mice exhibited liver tumors (hepatocellular neoplasms) at incidences greater than chamber controls but within the historical control incidence range.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	National Toxicology Program. (1999). Toxicology and carcinogenesis studies of ethyl-benzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (Inhalation studies) (Tech. Rep. Ser. No. 466; NIH Publ No. 99-3956), National Toxicology Program, U.S. Dept. of Health and Human Services. Research Triangle Park,

	NC. Brown, W.R. (2000). Ethylbenzene: Four week and 90-day inhalation toxicity studies in F344/N rats and B6C3F1 mice. IITRI Project Number L06206. Biodynamics Project Number 857853. Retrospective histopathologic evaluation of the kidneys (male and female rats), lung (male mice) and liver (female mice). Prepared for the Chemical Manufacturers Association, Ethylbenzene Panel, Arlington, VA.

[RS - 29]

Chronic Toxicity / Carcinogenicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.9 - 100%
Method:	Experimental (Non-regulatory)
Type:	Rodent Tumor Mode of Action Study
GLP:	Yes
Year:	2003
Species/Strain:	Rat / Fischer 344
Sex:	Males and Females
#/sex/dose:	Cell dynamics and histopathology subgroups - 6 animals/sex/dose for 1 week study and 8 animals/sex/dose for 4 week study Enzyme activity subgroups – 6 animals/sex/dose for 1 week study and 8 animals/sex/dose for 4 week study
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	Daily 6 hour exposures for 5 consecutive days for 1 week Daily 6 hour exposures, 5 days/week for 4 weeks
Doses/Concentrations:	75, 750 ppm Vapor - 1 week study 750 ppm Vapor - 4 week study
Control Group:	Control – Air only exposed
Statistical Methods:	All parameters examined statistically were first tested for equality of variance using Bartlett's test ($p = 0.01$). If the results from Bartlett's test were significant, then the data for the parameter were subjected to a transformation to obtain equality of variances. In the 1 week study, final body weight, organ weight (absolute and relative), clinical chemistry parameters, labeling index, apoptosis index, and enzyme assay data were evaluated using a 2-way ANOVA with the factors of sex and dose. If the sex-dose interaction was significant, a one-way analysis was done separately for each sex. If the dose effect was significant, comparisons of individual dose groups to the control group were made with Dunnett's tests. In the 4 week study, exploratory data analysis was performed by a

	parametric or nonparametric ANOVA. If significant, the ANOVA was followed by Dunnett's test or the Wilcoxon rank-sum test with a Bonferroni correction for multiple comparisons to the control.
Remarks for Test Conditions:	Animals, Maintenance, and Preparation - Species and strain: rat, Fischer 344(Charles River Laboratories, Raleigh, NC) - Sex: males and females. - Age: 7-8 weeks of age at the initiation of exposure - Acclimation period: 7 days - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum* except during exposure - Water: Municipal water, *ad libitum* except during exposure - The cell dynamic and histopathology subgroup animals had miniosmotic pups implanted for additional dosing of broodeoxyuridine (BrdU) during the entire exposure period for the 1 week study or for the forth week of exposure for the 4 week study. Chambers and Treatments Rats were exposed in 14.5 m^3 whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured by an automatic sampling system coupled to a gas chromatograph at least 6 times per exposure day in the 1 week study and 10-12 times per exposure period in the 4 week study. Target test article concentrations were 75 and 750 ppm. Assessments All animals were observed twice daily for moribundity and mortality. Body weights were recorded pre-study, on the first day of study prior to exposure, weekly thereafter (4 week study), and on the day of scheduled necropsy. *Necropsy, serum chemistry, histopathology, and electron microscopy.* All test animals were sacrificed on the day following the fifth or twentieth exposure for the 1 and 4 week studies, respectively. All animals were anesthetized with methoxyflurane, weighed, blood samples obtained by orbital sinus puncture, serum harvested, and serum-chemistry parameters assayed for alanine transainase, aspartate transaminase, alkaline phosphatase, creatinine, urea nitrogen, and γ-glutamyl transpeptidase. Animals were decapitated, exsanginated, and the kidneys excised and weighed. In the cell dyanamics and histopathology subgroup, sections of target tissues from 3 animals/tissue were collected and preserved in a 2% glutaraldehyde-2% formaldehyde fixative for electron microscopy. All remaining target tissues were immersion fixed in 10% neutral phosphate-buffered formalin. In the enzyme activities subgroup, kidneys were excised and snap frozen in liquid nitrogen and stored at -80ºC. Histologic sections of preserved kidneys were prepared by standard methods, stained with hemtoxylin and eosin and examined using light microscopy. The kidneys of male rats were examined using fluorescence microscopy. *S-phase DNA synthesis.* Levels of S-phase DNA synthesis were determined on serial sections of paraffin-embedded organs using

	an immunohistochemical technique for identification of BrdU incorporation into nuclear DNA outlined by Eldridge *et al*. (*Carcinogenesis* 11:2245-2251, 1990). BrDU labeled and unlabeled nuclei were counted from cortex (proximal convoluted tubules, outer medulla (inner and outer stripes), and inner medulla of kidneys. A labeling index (LI, the proportion of immunohistochemically stained nuclei to total nuclei), based on a minimum total count of nuclei (1000 in the cortex and outer stripe of the outer medulla and 300 in the inner stripe of the outer medulla and inner medulla), was calculated. Due to the focal nature of labeled nuclei in the renal cortex of the male rat, S-phase syntheses was subsequently revaluated by counting five cortical foci ("hot spots" having the highest concentration of labeled cells in a blinded manner. *Apoptosis*. Organs from high dose and control animals were processed and immunohistochemically stained for identification of apoptotic cells using ApopTag® Plus Kit. Stained and unstained cortical and medullary kidney cells were counted microscopically. An apoptosis index (AI, proportion of apoptotic cells) based upon a minimum total count of cells similar to those used for LI determination was calculated. *α-2u-globulin*. Deposition of α-2u-globulin in the kidneys of male rats was evaluated by immunohistochemical staining. *MFO and UGT Activities*. Microsomes from kidney were isolated and frozen until analyzed. CYP1A1, CYP1A2, CYP2B1/2 activities were measured *in vitro* as ethoxyresorufin (EROD), methoxyresorufin (MROD, four week study only) and pentoxyresorufin (PROD) *O*-dealkylase activities using fluorometric methods. Ethyoxyfluorocoumarin-O-dealkylase (EFCOD) activity providing a net activity of several MFOs, including CYP2E1, CYP1A and CYP2B was measured using a flurometric method. CYP2E1 activity was measured as para-nitrophenol (p-NPH) hydoxylase activity using a spectrophotometric method and UGT was measured using a spectrophotometric method.
Results:	NOEL – 75 ppm (1 week) Few changes were observed in the kidneys of rats exposed to 75 ppm ethylbenzene for 1 week.
Remarks:	<u>Chamber Concentrations</u> Actual mean exposure concentrations achieved in the chambers in the 1 week study were 75.2 and 738.6 ppm and in the 4 week study was 761 ppm ethylbenzene. *All results are presented by target inhalation level.* <u>Survival, Clinical Signs, Body Weights</u> There were no treatment-related effects on survival and clinical signs. Rats exposed to 750 ppm ethylbenzene weighed slightly less than controls; however, differences were statistically significant for both sexes only on study day 8 and for males on study day 27 and at 4 week necropsy.

	Serum Enzymes There were no effects from ethylbenzene exposure that were considered toxicologically significant for any of the serum enzymes or analytes measured. Pathology The kidney weights of males and females exposed to 750 ppm ethylbenzene were slightly increased (approximately 5-8%; p=0.05) following both exposure periods. The kidney weights of rats of either sex exposed to 75 ppm ethylbenzene in the 1 week study were not affected. Renal Histopathology, S-Phase DNA Synthesis, Apoptotic Activity and Enzyme Activity *Histopathology:* Histopathology examination of rat kidneys revealed an increase in number and size of hyaline droplets that occurred in the cells of the proximal tubules (PCT) of males exposed to 750 ppm ethylbenzene relative to controls in the 1 week study (2/6 very slight hyaline droplets and 2/6 slight hyaline droplets in controls versus 6/6 moderate hyaline droplets in 750 ppm males, respectively). Males exposed to 75 ppm ethylbenzene had only an equivocal increase in hyaline droplets in the PCT (1/6 very slight, 3/6 slight, and 1/6 moderate hyaline droplets in 75 ppm males). Following 4 weeks of exposure to 750 ppm ethylbenzene, male rats had a subtle renal lesion described as nephropathy, characterized by nuclear-size and staining variations and vacuolation or a decreased amount of cytoplasm (0/6 in controls and 2/6 multifocal, very slight and 6/6 slight nephropathy in 750 ppm males). This effect was present in multiple foci that were located primarily in the mid-cortical region, similar to the site and distribution of the areas where hyaline droplet accumulation is typically present in F344 male rats. No treatment-related renal changes were identified in females exposed to 750 ppm ethylbenzene in either study. *S-Phase DNA Synthesis:* Evaluation of S-phase DNA synthesis and α-2u-globulin deposition in 750 ppm males of both studies revealed localized focal effects in the cortical tubular epithelium, relative to controls. Changes coincided with foci of increased hyaline droplet deposition and tubular epithelial degeneration. Analysis of cortical "hot spots" revealed a 41% and 79% greater LI than controls in 1 and 4 week studies (p=0.05), respectively. Cortical "hot spots" also contained approximately 160% and 66% more α-2u-globulin than controls following 1 and 4 weeks exposure, respectively. There was no effect on cortical cell S-phase synthesis in males exposed to 75 ppm ethylbenzene for one week. The rate of S-phase DNA synthesis in the kidneys of female rats was lower than in males and labeled cells appeared randomly distributed in all areas examined. In the 1 week study, a nearly 50% decrease in LI was observed in cortical tubular epithelium of the 750 ppm females (p=0.05). In the 4 week study, no change in S-phase synthesis was observed in cortical tubular epithelium of exposed females; however, a minimal (28%) decrease was noted in synthesis rate in the outer stripe of the outer medulla. *Enzyme Activities:* There were relatively minimal changes in renal enzymes activities of exposed rats in both studies relative to

	controls. Para-NPH activity in males, and PROD activity in females, and UGT activity in both sexes of rats exposed to 750 ppm for 1 week were increased 89, 71, and 29-30% of control levels, respectively. There were no changes in enzyme activity levels of male and female rats exposed to 75 ppm ethylbenzene for 1 week. Following 4 weeks exposure, most enzyme activities were similar or slightly lower than control values, with the only statistically identified changes being an approximate decrease in the MROD and PROD activities in exposed females.
Conclusion:	Male rats exposed for 4 weeks to 750 ppm ethylbenzene exhibited increases in hyaline droplets, α-2u-globulin deposition, tubular epithelial degeneration, and S-phase DNA synthesis in proximal tubules. In female rats, only decreased S-phase synthesis and MFO activities occurred.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Stott, W.T., Johnson, K.A., Bahnemann, R., Day, S.J. and McGuirk, R.L. (2003). Evaluation of potential modes of actions of inhaled ethylbenzene in rats and mice. *Toxicol. Sci*. 71:53-66.

[RS - 30]

Chronic Toxicity / Carcinogenicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.9 - 100%
Method:	Experimental (Non-regulatory)
Type:	Rodent Tumor Mode of Action Study
GLP:	Yes
Year:	2003
Species/Strain:	Mouse / B6C3F1
Sex:	Males and Females
#/sex/dose:	Cell dynamics and histopathology subgroups - 6 animals/sex/dose for 1 week study and 8 animals/sex/dose for 4 week study Enzyme activity subgroups – 30 animals/sex/dose for 1 week study and 40 animals/sex/dose for 4 week study
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	Daily 6 hour exposures for 5 consecutive days for 1 week Daily 6 hour exposures, 5 days/week for 4 weeks
Doses/Concentrations:	75, 750 ppm Vapor - 1 week study 750 ppm Vapor - 4 week study
Control Group:	Control – Air only exposed
Statistical Methods:	All parameters examined statistically were first tested for equality of variance using Bartlett's test (p = 0.01). If the results from Bartlett's test were significant, then the data for the parameter were subjected to a transformation to obtain equality of variances. In the 1 week study, final body weight, organ weight (absolute and relative), clinical chemistry parameters, labeling index, apoptosis index, and enzyme assay data were evaluated using a 2-way ANOVA with the factors of sex and dose. If the sex-dose interaction was significant, a one-way analysis was done separately for each sex. If the dose effect was significant, comparisons of individual dose groups to the control group were made with Dunnett's tests. In the 4 week study, exploratory data analysis was performed by a

	parametric or nonparametric ANOVA. If significant, the ANOVA was followed by Dunnett's test or the Wilcoxon rank-sum test with a Bonferroni correction for multiple comparisons to the control.
Remarks for Test Conditions:	Animals, Maintenance, and Preparation - Species and strain: Mouse, B6C3F1(Charles River Laboratories, Raleigh, NC or Portage, MI) - Sex: males and females. - Age: 7-8 weeks of age at the initiation of exposure - Acclimation period: 7 days - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum* except during exposure - Water: Municipal water, *ad libitum* except during exposure - The cell dynamic and histopathology subgroup animals had miniosmotic pups implanted for additional dosing of bromodeoxyuridine (BrdU) during the entire exposure period for the 1 week study or for the forth week of exposure for the 4 week study. Chambers and Treatments Mice were exposed in 14.5 m^3 whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured by an automatic sampling system coupled to a gas chromatograph at least 6 times per exposure day in the 1 week study and 10-12 times per exposure period in the 4 week study. Target test article concentrations were 75 and 750 ppm. Assessments All animals were observed twice daily for moribundity and mortality. Body weights were recorded pre-study, on the first day of study prior to exposure, weekly thereafter (4 week study), and on the day of scheduled necropsy. *Necropsy, serum chemistry, histopathology, and electron microscopy.* All test animals were sacrificed on the day following the fifth or twentieth exposure for the 1 and 4 week studies, respectively. All animals were anesthetized with methoxyflurane, weighed, blood samples obtained by orbital sinus puncture, serum harvested, and serum-chemistry parameters assayed for alanine transainase, aspartate transaminase, alkaline phosphatase, creatinine, urea nitrogen, and γ-glutamyl transpeptidase. Animals were decapitated, exsanginated, and the livers and lungs excised and weighed. In the cell dyanamics and histopathology subgroup, sections of target tissues from 3 animals/tissue were collected and preserved in a 2% glutaraldehyde-2% formaldehyde fixative for electron microscopy. All remaining target tissues were immersion fixed in 10% neutral phosphate-buffered formalin. Mouse lungs were infused with fixative prior to immersion. In the enzyme activities subgroup, livers from 6 (1 week study) or 8 (4 week study) mice/sex/dose were excised and snap frozen in liquid nitrogen and stored at -80ºC. The lungs of mice from the enzyme groups were pooled to give 6-8 groups of 4 or 5 lungs each. Histologic sections of preserved livers and lungs were prepared by standard methods, stained with hemtoxylin and eosin and examined using light microscopy.

	S-phase DNA synthesis. Levels of S-phase DNA synthesis were determined on serial sections of paraffin-embedded organs using an immunohistochemical technique for identification of BrdU incorporation into nuclear DNA outlined by Eldridge *et al*. (*Carcinogenesis* 11:2245-2251, 1990). BrDU labeled and unlabeled nuclei were counted from hepatocytes from the centrilobular, midzonal, and periportal regions of mouse livers and epithelial cells of the lower airways and alveoli of mouse lungs. A labeling index (LI, the proportion of immunohistochemically stained nuclei to total nuclei), based on a minimum total count of nuclei (2000 in each of the regions of the liver and 1000 in the smaller airways and alveoli of the lung), was calculated. Liver sections were evaluated using the lobule-dependent zonal measurement method outlined by Bahnemann and Mellert (*Toxicol. Path*. 49:189-196, 1997) by use of an ocular grid at 250x total magnification. *Apoptosis*. Organs from high dose and control animals were processed and immunohistochemically stained for identification of apoptotic cells using ApopTag® Plus Kit. Stained and unstained centrilobular and periportal mouse liver hepatocytes and epithelium of the lower airways and alveoli of the mouse lung were counted microscopically. An apoptosis index (AI, proportion of apoptotic cells) based upon a minimum total count of cells similar to those used for LI determination was calculated. *MFO and UGT Activities*. Microsomes from livers and lungs were isolated and frozen until analyzed. CYP1A1, CYP1A2, CYP2B1/2 activities were measured *in vitro* as ethoxyresorufin (EROD), methoxyresorufin (MROD, four week study only) and pentoxyresorufin (PROD) *O*-dealkylase activities using fluorometric methods. Ethyoxyfluorocoumarin-O-dealkylase (EFCOD) activity providing a net activity of several MFOs, including CYP2E1, CYP1A and CYP2B was measured using a flurometric method. CYP2E1 activity was measured as para-nitrophenol (p-NPH) hydoxylase activity using a spectrophotometric method and UGT was measured using a spectrophotometric method.
Results:	NOEL – 75 ppm (1 week) Few changes were observed in the livers and lungs of mice exposed to 75 ppm ethylbenzene for 1 week.
Remarks:	<u>Chamber Concentrations</u> Actual mean exposure concentrations achieved in the chambers in the 1 week study were 75.2 and 738.6 ppm and in the 4 week study was 761 ppm ethylbenzene. *All results are presented by target inhalation level.* <u>Survival, Clinical Signs, Body Weights</u> There were no treatment related effects on survival and clinical signs. Some spontaneous deaths occurred early in the study. In the 1 week study, 3 control females and 2 of the 75 ppm group males died while 7 females from the 750 ppm group died in the 4 week study, all within the first few days of exposure. These animals were diagnosed as cachexic, apparently the result of poor adaptation to

	the inhalation-chamber environment. There were no statistically identified differences in the body weights of any exposure group relative to controls. Serum Enzymes There were no effects from ethylbenzene exposure that were considered toxicologically significant for any of the serum enzymes or analytes measured. Pathology (Gross and Organ Weights) There were no gross pathologic effects identified at necropsy that were related to the inhalation exposure of mice to up to 750 ppm ethylbenzene in either study. After both exposure periods, the absolute and relative liver weights were increased approximately 6-12% and 13-16% in 750 ppm males and females, respectively ($p=0.05$). Liver weight was not affected in mice of either sex exposed to 75 ppm ethylbenzene for 1 week. There was no effect of ethylbenzene exposure upon lung weights in either sex of mouse at either necropsy time point. Liver and Lung Histopathology, S-Phase DNA Synthesis, Apoptotic Activity and Enzyme Activity *Histopathology*: Histopathology examination of livers of mice exposed to 750 ppm ethylbenzene found increased numbers of mitotic figures in the majority of exposed males and females, most in the midzonal to centrilobular areas, in both studies. More mitoses were present in the liver of control and 75 ppm ethylbenzene exposure group females than males. There were no histopathologic effects related to ethylbenzene exposure noted in the lungs of mice of either sex or study. Additional evaluation of mouse liver and lung using electron microscopy revealed no significant treatment-related changes at an ultrastructural level. *S-Phase DNA Synthesis*: The relative rate of S-phase DNA synthesis in the liver of male mice of the 1 week study exposed to 750 ppm ethylbenzene progressively increased across the liver lobule from a 180% increase in periportal hepatocytes to a 479% increase in midzonal hepatocytes to a 1116% increases in centrilobular hepatocytes. The differential anatomic effect was still evident but less pronounced after 4 weeks of 750 ppm exposure. A similar regional progression was evident in 750 ppm females from the 1 week study. Females had higher LI levels in all hepatic zones than males, however, the relative increase was less due to a much greater control LI level. A relatively high LI was also observed in 4 week study females, however a relatively high degree of interanimal variability confounded interpretation. A maximal 56% increase in LI was noted in centrilobular hepatocytes of female mice exposed to 750 ppm ethylbenzene. Only minimal, nonsignificant changes in LI were noted in either sex of mice exposed to 75 ppm ethylbenzene for 1 week. *Apoptosis*: In the lungs of 750 ppm mice there appeared to be a consistent increase in BrdU-labeled cells in the smaller airways (terminal bronchioles) relative to controls. In the 1 week study, LI were increased 180% and 149% over controls in males and females, respectively. After 4 weeks exposure, the LI of 750 ppm

	males and females was increased 82 and 115% over controls, respectively, but not significantly different. No consistent effect upon S-phase synthesis was found in the alveoli. A statistically significant decrease of 38% in the S-phase synthesis rate of alveolar epithelium was found in the ethylbenzene exposed males after 4 weeks; however alveolar cell LI was not affected either after 1 week nor for exposed females at either time period. Evaluation of apoptosis in liver and lungs from control and high exposure groups form the 1 week study revealed only infrequent apoptotic cells, usually 0 to 1 cells per anatomic region. *Enzyme Activities*: A number of treatment-related changes were found in enzyme activities. In the mouse liver, following 1 week of ethylbenzene exposure, EROD activity was minimally elevated approximately 40-60% in 750 ppm males and females relative to controls. In male mice, similar increases were also noted in PROD and in EFCOD. Female mice exhibited a small increase in PROD activity that, although statistically significant, was considered inconsequential. Treatment-related alterations of EFCOD and p-NPH were not observed in female mouse liver nor was altered UGT activity in the liver of any exposed mouse. At 75 ppm ethylbenzene, the PROD and EFCOD activities were slightly but statistically significantly decreased for both sexes of mice. Following 4 weeks of exposure to 750 ppm ethylbenzene, liver PROD activity remained statistically increased in males (81%) and females (130%) relative to controls. In females, EROD and UGT were also significantly increased by 61 and 31%, respectively. Minimal 24-27% increases, but not statistically significant, in mean p-NPH activity were also noted in both sexes. In the mouse lung, following one week of ethylbenzene exposure, the *in vitro* activities of several MFO enzymes was decreased in a dose-related manner relative to controls. The activities of EROD, PROD, EFCOD were decreased 17-33% in males and females inhaling 75 ppm ethylbenzene and 25-45% in both sexes inhaling 750 ppm ethylbenzene. No significant net changes in *in vitro* pulmonary p-NPH was observed in treated animals. After 4 weeks of exposure, lung metabolic enzymes of males and females differed in their response to inhaled ethylbenzene. In males, the activities of p-NPH and UGT were statistically increased by 73 and 51%, respectively, relative to controls. In females, the activities of EROD, MROD and PROD were statistically decreased 33-50%.
Conclusion:	Mice exposed to 750 ppm ethylbenzene vapor for 4 weeks exhibited increased liver weights, hepatocellular hypertrophy, mitotic figures, S-phase DNA synthesis and enzyme activities. S-phase synthesis rates in terminal bronchiolar epithelium were elevated and accompanied by loss of MFO activity.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Stott, W.T., Johnson, K.A., Bahnemann, R., Day, S.J. and McGuirk, R.L. (2003). Evaluation of potential modes of actions of inhaled ethylbenzene in rats and mice. *Toxicol. Sci*. 71:53-66.

[RS - 31]

Neurotoxicity Screening Battery: Acute Neurotoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99%
Method:	Experimental (Non-regulatory); Functional Observational Battery Protocol generally conformed with US EPA Guidelines (adapted for the mouse)
Type:	Acute Neurotoxicity
GLP:	Not Specified
Year:	1994
Species/Strain:	Mouse / CFW
Sex:	Male
#/dose:	8
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	20 minutes, Single Exposure
Doses/Concentrations:	2000, 4000, 8000 ppm - Vapor
Control Group:	Positive control – Pentobarbital, intraperitoneal injections of 5 – 40 mg/kg bwt Negative Control – Air only
Remarks for Test Conditions:	Animals and Maintenance - Species and strain: albino mice, CFW (ChasRiver Swiss) (Charles River Breeding Laboratories, North Wilmington, MA) - Sex: males - Age: adult, specific age not specified - Body weight: approximately 25-30 g at the time of testing - Acclimation period: not specified - Housing: individually in plastic cages fitted with steel wire tops - Environment: temperature controlled to 22-24ºC - Diet: not specified - Water: not specified Chambers Static vapor exposures were carried out in 29 L clear cylindrical

	glass jars. Exposures were accomplished by injecting a predetermined amount of liquid test material through an injection port in the jar lid onto filter paper placed on a platform within the jar and turning on a fan located in the lid. The nominal vapor concentrations were confirmed by single wavelength monitoring infrared spectrometry. Assessment A functional observational battery (FOB) protocol was performed on mice during and immediately after exposure. Observations were carried out by blinded observers and results were scored using a standardized procedure. During the last 2 minutes of the inhalation exposure, mice were scored on eight measures (posture, arousal, rearing, clonic movements, tonic movements, palpebral closure, gait and gait abnormalities). Following exposure, mice were removed from the exposure chamber within 10-15 seconds and evaluated on the complete FOB, using the general procedure by Moser *et al.* (*Fundam. Appl. Toxicol.*, 11-189-206, 1988). The duration of the open field assessment was 2 minutes and a 20 second cutoff was used for the inverted screen test. The entire evaluation required 3-4 minutes per mouse. The evaluation of pentobarbital utilized a somewhat different procedure, and the scoring of a few of the measures in the FOB was slightly different from that used for the test materials. Evaluation of Results/Statistical Analyses Multiple comparisons were made between each level of the test material compared to the combined control results (n=16) for each measure. All quantal, descriptive and rank-order data were tested for significance by the nonparameteric *z* test for significant differences between proportions. Interval data were test for significant differences from control using a two-tailed unpaired Student *t* test. Bonferroni's procedure was used to adjust the α level for each comparison so that the $p \leq 0.05$ for the set of comparisons.
Results:	LOAEL (Concentration altering FOB) – 2000 ppm
Remarks:	Chamber Concentrations No information provided on actual exposure concentrations. FOB Assessment During exposure, ethylbenzene produced dose-dependent (at 2000, 4000, and 8000 ppm) abnormal postures and decrease in arousal and rearing ($p \leq 0.05$). Statistically significant changes were also present in palpebral closure, gait and gait abnormalities ($p \leq 0.05$). After exposure, significant changes ($p \leq 0.05$) were present (affected doses noted where reported), on arousal (decreased), rearing (decreased only at 8000 ppm), ease of removal from chamber (increased), lacrimation (increased), gait and gait abnormalities, mobility (decreased), righting reflex (decreased at all doses), forelimb grip strength (decreased at all doses), inverted screen (decreased at 4000 and 8000 ppm), landing foot splay (increased at all doses), approach response (decreased), click response (decreased), touch response (decreased), and tail pinch response (decreased).

Conclusion:	Ethylbenzene produced neurobehavioral changes in mice during and shortly after receiving 20 minute exposures to ≥ 2000 ppm.
Data Quality:	2 – Reliable study with restrictions. Study is sufficiently documented and meets generally accepted scientific principles.
Reference:	Tegeris, J.S. and Balster, R.L. (1994). A comparison of the acute behavioral effects of alkylbenzenes using a functional observational battery in mice. *Fundam. Appl. Toxicol.* 22:240-250.

[RS - 32]

Neurotoxicity Screening Battery: Acute Neurotoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99%
Method:	Experimental (Non-regulatory)
Type:	Acute Neurotoxicity
GLP:	Not Specified
Year:	1986
Species/Strain:	Rat / CFY
Sex:	Male
#/dose:	8
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	1 to 4 hours
Doses/Concentrations:	At least 6 concentrations (between 100 and 3000 ppm), up to concentrations inducing anesthesia
Control Group:	Yes
Remarks for Test Conditions:	Animals and Maintenance - Species and strain: rat, CFY (LATI, Godollo, Hungary) - Sex: males - Age: 8 weeks at the time of testing - Body weight: approximately 200 g at the time of testing - Acclimation period: not specified - Housing: in groups of 10 in wire mesh cages - Environment: not specified - Diet: standard rat diet of pellets, *ad libitum* - Water: tap water, *ad libitum* Chambers Rats were exposed in 30 L cylindrical glass chambers. Exposure concentrations were determined at 30 minute intervals by an ultraviolet spectrophotometric technique. Assessments Rats were assessed for group motility during exposure using 4

	electromechnical transducers built into metal tubes which were fixed in a perpendicular position within the exposure chamber. The moving rats operated the transducers by touching the tubes and the number of touchings was recorded continuously by an electric counter outside the chamber. No information was collected on clinical signs during or after exposure or body weight changes. Evaluation of Results/Statistical Analyses No statistical analyses performed.
Results:	Minimum narcotic concentration - 2180 ppm Concentration altering motor behavior – 400 – 1500 ppm (moderate activation)
Remarks:	Chamber Concentrations No information provided on actual exposure concentrations. Motor Activity Assessment Ethylbenzene produced a bell-shaped concentration action curve characteristic of a biphasic effect (*i.e.,* activation at lower and depression at higher concentrations). Moderate activation in rat motor activity was apparent in the range of 400 to 1500 ppm after 4 hours of exposure and a decrease in motor activity was apparent at approximately 2000 and greater ppm ethylbenzene. The authors calculated that the minimum narcotic concentration for ethylbenzene following 4 hours of exposure was 2180 ppm.
Conclusion:	The minimum narcotic concentration for ethylbenzene in rats is 2180 ppm.
Data Quality:	4 – Not assignable. Actual exposure measurements not reported. Statistical analyses not performed. Non standard assessment of motor activity.
Reference:	Molnar, J., Katalin, A. and Naray, M. (1986). Changes in the rat's motor behaviour during 4-hr inhalation exposure to prenarcotic concentrations of benzene and its derivatives. *Acta Physiologica Hungarica.* 67(3):349-354.

[RS - 33]

Neurotoxicity Screening Battery: Subchronic Neurotoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.96%
Method:	EPA Health Effects Testing Guidelines OPPTS 870.6200; Generally conforms with OECD 424 Guideline for Neurotoxicity Study in Rodents with exception that behavioral tests were not conducted during the first or second week of exposure.
Type:	Subchronic (90-day) Repeated Dose Oral Neurotoxicity Study
GLP:	Yes
Year:	2006
Species/Strain:	Rat / Sprague-Dawley
Sex:	Male and Female
#/sex/dose:	16 (control and high dose); 10 (low and mid dose)
Vehicle:	Corn oil
Route of Administration:	Oral Gavage
Exposure Period and Frequency of Treatment:	Daily for 91 consecutive days Daily dosage was divided into two doses administered to each rat approximately 3 hours apart. Each portion was administered beginning at approximately 12:30 and 15:30 daily. The first daily dose was administered after completion of behavioral tests.
Doses/Concentrations:	Daily dosage: 50, 250, 500 mg/kg bwt/day
Control Group:	Yes
Statistical Methods:	<u>Body weights, Feed Consumption, Organ Weights:</u> Bartlett's test of homogeneity of variances was used first to estimate the probability that the groups have different variances. If the test was not significant (p>0.001), the data were compared using a one-way analysis of variance test (ANOVA). If the ANOVA was significant (p≤0.05), the control group was compared with each other treatment group using Dunnett's test. If Bartlett's test was significant (p≤0.001), the data were evaluated using nonparametric procedures. The Kruskal-Wallis test was used as the nonparametric test when 75% or fewer of the scores in all the groups are tied, and in the event of a significant result (p≤0.05), Dunn's test was used to compare the control group with each other

	treatment group. When more than 75% of the scores in any group are tied at a single value, Fisher's exact test was used as the nonparametric test. **Functional Observational Battery and Motor Activity** Graded, Quantal and Count Data Endpoints Analyses of graded (e.g., reactions to handling and other stimuli, quantal (e.g., presence or absence of piloerection) and count (e.g., rears in the open field) data endpoints were conducted, by sex, for each testing session with the extended Mantel-Haenszel (MH) method. If the test was significant at the 0.05 significance level, the analysis was repeated excluding the highest dosage group. The procedure continued until the test was not significant. Continuous Data Endpoints (except within session motor activity): Predosage measurements of continuous data endpoints (e.g., grip tests, landing foot splay, total session motor activity) were analyzed with an ANOVA. Analyses of continuous data endpoints for the FOB and total session motor activity data during the dosage period were conducted with a repeated measure analysis of covariance (RANCOVA). The SAS® procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by evaluating Akaike's Information Criterion (AIC). If any of the interaction terms including DOSE*SEX were significant the analysis was conducted, by sex, with a RANCOVA including the following terms: DOSE, SESSION, DOSE* SESSION, and PREDOSE as the covariate. In the final model, monotonicity of dosage response was examined using sequential trend tests based on ordinal spacing of dosage levels. Dosage-response trend tests on group means were preceded by two linear treatment by session interaction tests: 1) linear trend in dosage by linear trend in session (LinDOSE*LinSESSION); and 2) linear trend in dosage by quadratic trend in session (LinDOSE*QdrSESSION). If either of the two interactions were significant at the 0.05 significance level, the trend tests were performed for each session. If neither interaction was significant the trend tests were performed across the pooled sessions. Motor activity intersession data. For the pretreatment testing session, an ANOVA was conducted. For testing sessions conducted during the treatment period, each endpoint was analyzed using a repeated measure analysis of variance (RANOVA). Factors in the model included DOSE, SEX, time interval (TIME) within the session, and the following interaction terms: DOSE*SEX, DOSE*TIME, and DOSE*SEX*TIME. The SAS® procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by evaluating

	Akaike's Information Criterion (AIC). If any of the interaction terms including DOSE*SEX were significant, the analysis was conducted separately for each sex with a RANOVA including the following terms: DOSE, TIME and DOSE*TIME. In the final model, two linear dosage by time interactions were evaluated at the 0.05 significance level: 1) linear trend in dosage by linear trend in time (LinDOSE*LinTIME); and 2) linear trend in dosage by quadratic trend in time (LinDOSE*QdrTIME). If neither interaction is significant, monotonicity of dose response was examined for the session as a whole using sequential trend tests based on ordinal spacing of dose levels. If either of the two interactions were significant, the same two linear treatment by time interactions were evaluated excluding the highest dose group. The sequential testing (excluding the highest dose group) of the two linear treatment by time interactions continued until neither was significant. Neurohistological Examination The incidences of microscopic alterations were analyzed for each sex using one-tailed Fisher's exact tests comparing the incidence in the control group with that for each other dosage group examined, in order to determine the statistical significance of increased incidences with treatment.
Remarks for Test Conditions:	Animals and Maintenance – Species and strain: rat, Sprague-Dawley [Crl:CD(SD) Charles River Laboratories, Inc., Kingston, New York – Age on receipt: approximately 41 days – Acclimation period: 5 days – Housing: individually housed in suspended stainless-steel wire-mesh cages. – Diet: Certified Rodent Diet® #5002 (PMI® Nutrition International, Inc., St. Louis, Missouri), access *ad libitum* – Water: access *ad libitum* – Environment: Temperature averaged 19-25°C; Relative Humidity averaged 30-70% Dose Selection: The selection of the top dose of 500 mg/kg bwt/day (250 mg/kg bwt 2X/day, 3 hours apart) was based on the results of previous repeated dose toxicity studies and PBPK modeling comparing oral doses with inhalation exposures. Preparation of Dosing Solutions Oral dosing solutions were prepared weekly in corn oil vehicle and stored at room temperature for a period that did not exceed 9 days in duration. Analysis of Dosing Solutions The test substance samples prepared during weeks 1, 4, 8 and 13 were all within 10% of the target levels for the 2.5, 12.5 and 25 mg/mL dosage concentrations Stability was determined over 9 days (room temperature) for the low and high dosage group solutions.

	Viability and Clinical Observations Rats were observed for viability at least twice each day of the study. Clinical observations were recorded prior to each of the two daily doses and daily after the end of the dosage period. Body Weight and Feed Consumption Body weights and feed consumption were recorded once weekly during the dosage period and on the day of sacrifice. Body weights were also recorded on the days that behavioral tests were conducted. Detailed Clinical Observations Detailed clinical observations were conducted weekly during the dosage period with the exception of those weeks on which the functional observational battery (FOB) was scheduled. The clinical observations were conducted without knowledge of treatment level by observers certified to conduct the FOB. Ophthalmological Examinations Ophthalmological examinations were performed by a veterinary ophthalmologist for all rats prior to dosage and within 1 week of scheduled sacrifice. Neurobehavioral Assessment A functional observational battery (FOB) and motor activity test were conducted before the first day of dosing and during week 4, 8 and 13 of the dosage period. During each of the four test sessions, the behavioral tests were conducted on the rats over four days. The rats were divided into four replicates such that approximately 26 rats were tested each day. Dosage groups and gender were counterbalanced across the four replicates. The motor activity and FOB evaluations were conducted prior to daily dosing at approximately the same time of day for all of the test sessions. The FOB was conducted by a single trained observer unaware of the group assignment of each rat. Evaluations were conducted in the home cage, during handling the rat, for a 2-minute period in an open field and following reactivity and sensitivity tests. The FOB evaluation included the following parameters: 1. Lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, and urination and defecation (autonomic functions). 2. Sensorimotor responses to visual, acoustic, tactile and painful stimuli (reactivity and sensitivity). 3. Reactions to handling and behavior in the open field (excitability). 4. Gait pattern in the open field, severity of gait abnormalities, air righting reaction, visual placing response and landing foot splay (gait and sensorimotor coordination). 5. Forelimb and hindlimb grip tests.

	6. Detailed clinical observations as described previously including, but not limited to, abnormal clinical signs such as convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance and deposits around the eyes, nose or mouth. 7. Body weight and body temperature. Each motor activity test session was 1 hour in duration with time spent in movement and the number of movements tabulated at each 10-minute interval.The movements of each rat were monitored by a passive infrared sensor (Coulbourn Instruments Passive Infrared Motor Activity System) mounted outside a stainless steel, wire-bottomed cage. The dosage groups were counterbalanced across testing sessions and cages. <u>Gross Necropsy and Histopathology</u> The rats were sacrificed after the end of the dosage period. Nine to 11 rats/sex/dosage group were anesthetized and then perfused *in situ* with neutral buffered 10% formalin. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed, and the liver and kidneys were weighed. The brains of rats selected for neurohistological examination were weighed. The liver, kidney and nervous system tissues of all the perfused animals in all dosage groups were retained. The kidneys and livers from all perfused animals in the control and 500 mg/kg bwt/day dose group were examined histologically. The eyes, brain, spinal cord, and hindlimb peripheral nerves and muscle from 6 of the rats perfused in the control male dosage group, 7 in the female control dosage group and 6in the male and female high dosage groups were examined histologically. The remaining rats in the control and high dosage groups that were not selected for perfusion fixation were sacrificed by carbon dioxide asphyxiation and a gross necropsy was performed. The nervous system tissues processed for histological evaluation were the cervical and lumbar regions of the spinal cord (including the cervical and lumbar enlargements of the spinal cord and ventral and dorsal root ganglia and nerve roots), the sural nerve and sections of the sciatic nerve (mid-thigh), tibial nerve (proximal, in the knee area and the gastrocnemius bifurcation) and skeletal muscle (gastrocnemius) from the selected rats were removed and, along with the gassarian ganglion, brain (forebrain, central cerebrum with midbrain, cerebellum with pons, and medulla oblongata) and the eyeball (including the optic nerve and retina). Sections from the spinal cord and peripheral nerves included both longitudinal and transverse sections. The central nervous system tissues, muscle, liver and kidneys were embedded in paraffin, and the peripheral nerves, including the ganglia and spinal nerve roots, were embedded in plastic. Sections were stained with hematoxylin and eosin. Additional sections from tissues embedded in paraffin (with the exception of the muscle, liver and kidneys) were stained with luxol fast blue/cresyl violet, and sections from tissues embedded in plastic were stained with toluidine blue.

Results:	Systemic Toxicity NOEL - 50 mg/kg bwt/day based on increase in liver and kidney weights. Adult Neurotoxicity NOEL - 500 mg/kg bwt/day.
Remarks:	Dose confirmation All analytical results were within the acceptable limits: within 10% of the target for concentration values, relative standard deviations of ≤5% for homogeneity evaluations, and within 10% of the baseline value for stability evaluations. *All results are presented by target dosage level.* Mortality One male rat and one female rat at 250 mg/kg bwt/day were sacrificed in moribund condition on days 71 and 50 of the study, respectively and one male rat at 500 mg/kg bwt/day was found dead on study day 88. These deaths were considered incidental events unrelated to the test substance because the frequency was not dosage-dependent, and the death at 500 mg/kg bwt/day appeared to be associated with injury to the snout. All other rats survived until scheduled sacrifice. Body Weight and Feed Consumption Significant changes in body weight gains and/or feed consumption values occurred at several weekly intervals in the groups given 250 or 500 mg/kg bwt/day of ethylbenzene, but values for the entire dosage period (calculated as study days 1 to 92) were generally comparable to the controls. Body weight gains in the male rats at 250 and 500 mg/kg bwt/day were decreased 12.7% and 7.8%, respectively, for this period as compared with the control group value. The respective values for the female rats at 250 and 500 mg/kg bwt/day were 16.2% and 15.3% higher than the control group. Absolute feed consumption values (g/day) for the dosage period were increased 1.3% and 7.4% in the males at 250 and 500 mg/kg bwt/day, respectively, and the increases in the females in these groups were 8.4% and 12.0%. The increases in relative feed consumption (g/kg bwt/day) were 6.1% and 12.2% in the males and 5.1% and 7.0% in the females at 250 and 500 mg/kg bwt/day. Clinical Observations At 500 mg/kg bwt/day, there were slight increases in the numbers of male and female rats observed with slight to moderate excess salivation and marginal increases in urine-stained abdominal fur. The majority of observations of excess salivation occurred around the time that the daily doses were administered. Urine-stained abdominal fur was increased in all the dosage groups and was frequently evident prior to the first of the two daily doses. Ophthalmological Observations There were no adverse observations apparent during the ophthalmological examination conducted at the end of dosage administration period.

	Functional Observation Battery (FOB) There were no differences between the dosage groups in a large majority of the FOB measures. In the first session conducted during the dosage period (Week 4), significantly more female rats at 500 mg/kg bwt/day ($p \leq 0.01$, 5 of 16 as compared with 0 of 16 in the control group) had normal levels of urination during the open field evaluation. The rats that did not have "normal" levels of urination had no urination. None of the rats had "excess urination". This difference is not considered to be a toxicologically relevant effect because (a) the observation was that of "normal" level of urination, (b) there were no differences among the groups in this parameter in the subsequent test sessions as exposure to the test substance increased, and (c) the incidence was within the range of the control values recorded during pre-test and later time periods. In the evaluation conducted during Week 8 of the dosage period, the acoustic stimulus elicited a startle reaction in significantly more ($p \leq 0.05$) female rats at 500 mg/kg bwt/day, whereas more control rats displayed an orienting response (all 16 startled at 500 mg/kg bwt/day compared to 4 oriented and 12 startled in controls). In the subsequent evaluation during Week 13, significantly fewer ($p \leq 0.05$) male rats at this dosage displayed a startle reaction while all controls reacted to the stimulus with a startle response (3 oriented and 13 startled from the 500 mg/kg bwt/day group compared to all 16 startled in controls). These differences were considered incidental events because (a) both behaviors are commonly observed as normal reactions to the acoustic stimulus, (b) the differences were similar to the control or predosage values and (c) differences were not consistent across sessions or between the male and female rats. Motor Activity No significant differences among the dosage groups for both the number of movements and time spent in movement were revealed by analyses of the values across the test sessions during the dosing period. The cumulative values for number of movements and total time spent in movement at each session were compared, and there were no dose-related changes in the pattern of these values during the dosing period nor were there dose-related differences between the averages calculated across the test sessions. In addition to the repeated measures analysis, two linear trend analyses (LinDOSE*LinTIME and LinDOSE*QdrTIME) were conducted within the framework of the repeated measures analysis to evaluate the effect of treatment on the within session activity. At week 4, there were no statistically significant differences in the LinDOSE*QdrTIME trend for the number of movements and time spent in movement within each session. However, there was a statistically significant difference in the LinDOSE*LinTIME for the time spent in movement but not the number of movements at 250 and 500 mg/kg bwt/day. The mean time spent in movement within the session was similar for all dosage groups except for just the last 10 minutes of the test session, where activity was higher in the 250 and 500 mg/kg bwt/day dosage groups. This is not considered

	treatment-related because (a) there was no clear dose-related pattern in the averages (the values for the 250 mg/kg bwt/day dosage group generally exceeded those of the 500 mg/kg bwt/day and values for the 50 mg/kg bwt/day dosage group were generally lower than the control values); and (b) there were no statistically significant differences among the dosage groups in the measurements for motor activity after longer exposure durations of 8 and 13 weeks. Necropsy Observations Few gross lesions were observed in the male and female rats at necropsy, and none were considered test substance-related. Absolute brain weights in the male and female rats were unaffected at the highest dose level of 500 mg/kg bwt/day. The significant increase ($p \leq 0.01$) in the ratio of brain weight to terminal body weight is attributed to the slight decrease in terminal body weight that occurred in the 500 mg/kg bwt/day dosage group. The absolute organ weights for the liver and paired kidneys were increased for both the male and female rats at 250 and 500 mg/kg bwt/day, and relative weights of these organs to terminal body weights were increased or significantly increased ($p \leq 0.05$ to $p \leq 0.01$) in both male and female rats at the 250 and 500 mg/kg bwt/day dosage groups. These kidney and liver weight changes are not expected with reductions in body weight alone and are considered effects of ethylbenzene. Histopathology Evaluation The histological examination of tissues from the control and 500-mg/kg bwt/day dosage group male and female rats did not reveal any test substance-related microscopic lesions in the neural and muscle tissues evaluated nor in the livers and kidneys. The alterations that were observed were considered spontaneous background lesions. The only lesion observed in the neurohistological examination was minimal nerve fiber degeneration in the trapezoid body, which was identified in one control group rat and a single rat at 500 mg/kg bwt/day. Circumscribed vacuoles (fatty metamorphosis) in the liver were observed in significantly more males ($p \leq 0.05$) at 500 mg/kg bwt/day as compared with the controls (9 versus 5); however, the severity of findings did not increase with dosage, and the incidence among the females were high for both groups (9 and 8 rats in the control and 500 mg/kg bwt/day groups, respectively). Minimal to mild degrees of inflammatory cell infiltrates, which are common at these degrees of severity, were also observed in livers of rats from both groups. One male rat at 500 mg/kg bwt/day had pyelonephritis, which was considered a background lesion, as were the low incidences of hydronephrosis, tubular mineralization, protein filled tubules, tubular ectasis, interstial nephritis, and tubular cell hyperplasia. Recovery Group Additional rats were included in the control and 500 mg/kg bwt/day dosage group so that an optional recovery group could be available if behavioral effects were noted. Since there were no treatment-related effects on the FOB or motor activity during exposure, some of the animals from the recovery group were used to increase the

	number of animals assigned for histopathology evaluation of the kidney and liver from 6 rats/sex/dose to 10 rats/sex/dose. Postive Control Data Historical positive control data for motor activity, functional observation battery and neuropathology demonstrated the ability of procedures and trained personnel to detect chemically-induced effects
Conclusion:	Repeated 91-day oral exposure of young adult rats to ethylbenzene at dosages ranging from 50 to 500 mg/kg bwt/day did not cause treatment-related effects on the FOB, motor activity, and histopathology examination of the central and peripheral nervous system. The 250 and 500 mg/kg bwt/day dosage of ethylbenzene increased liver and kidney weights in male and female rats. In addition, the 500 mg/kg bwt/day dosage increased incidences of clinical signs in both males and females and increased relative feed consumption in the males.
Data Quality:	1- Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant results.
Reference:	Barnett, John F. Jr. (2006). Oral (Gavage) subchronic neurotoxicity study of ethylbenzene in rats with recovery group. Laboratory Project ID MHV00001. Charles River DDS Argus Division Laboratory, Horsham, Pennsylvania. Sponsored by the Ethylbenzene Panel, American Chemistry Council, Arlington, VA.

[RS - 34]

Neurotoxicity Screening Battery: Subchronic Ototoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99%
Method:	Experimental (Non-regulatory)
Type:	Subchronic Ototoxicity Study
GLP:	Not Specified
Year:	2006
Species/Strain:	Rat / Sprague-Dawley
Sex:	Male
#/dose:	14
Vehicle:	Air
Route of Administration:	Inhalation
Exposure Period and Frequency of Treatment:	6 hours/day, 6 days/week for 13 weeks 8 week post exposure recovery period
Doses/Concentrations:	200, 400, 600, 800 ppm – Vapor
Control Group:	Yes
Statistical Methods:	The data of the continuous, parametric variables (body weights) were compared for the exposure and control groups by use of Bartlett's test for homogeneity of variances and by analysis of variance. Repeated measurement ANOVAs were not used because data were not available for all the rats (due to lost head plugs or deaths) throughout the experiment. If the analysis of variance was significant, individual mean comparisons were made with Scheffe's multiple range test to make comparisons between any pair of groups. Non-parametric data (audiometric thresholds) were statistically evaluated using the non-parametric Kruskal-Wallis test. The probability value of $P<0.05$ was used as the critical level of significance. The percentages of cell losses in the third row of the Outer Hair Cells (OHC) and the test substance's concentrations were fitted using logistic regression with independent variables log (concentration) using a non-linear regression program (STATA 8, College Station, Texas). This regression analysis was used to calculate the theoretical concentration causing 50% loses in the

	third row of the OHC (EC50). In the absence of a no observed adverse effect level (NOAEL), theoretical lowest adverse effect levels (TLAEL) were calculated from the statistical upper confidence limits (95, 99, 99.9%) of the average losses observed in the controls (mean + 1.96, 2.58 and 3.29 SD, respectively).
Remarks for Test Conditions:	Animals and Maintenance Species and strain: rat, Sprague-Dawley (Iffa Credo, Domaine des Oncins, Saint-Germain-sur-l'Arbresle, France) Sex: male Age on receipt: 13 weeks old Acclimation period: 6 days Housing: individually housed in polypropylene cages in woodchip bedding Diet: UAR-Alimentation (Villemoisson, Epinay-sur Orge, France) sterilized with gamma rays, access *ad libitum* Water: filtered tap water (pore size 0.3µm), access *ad libitum* Environment: controlled to 22 ºC, 55 ± 5% relative humidity, 12 hour light/dark cycle Chamber Conditions Rats were exposed in 200-L stainless steel inhalation chambers designed to maintain a dynamic and adjustable airflow (4-30 m^3/hour) The control groups were exposed in the same conditions to clean filtered air. An additional airflow was bubbled through ethylbenzene and the output vapor was diluted with air to the required concentration before entering the exposure chambers Concentrations of ethylbenzene in the inhalation chambers were determined once every 6 hour exposure period using a gas chromatograph. Treatment Five groups of male Sprague-Dawley rats (14/group) were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, for 6 hours daily, 6 days/week for 13 weeks. Target test article concentrations were 0, 200, 400, 600 and 800 ppm. The animals were maintained for a recovery period of 8 weeks following exposure before being sacrificed. Ambient sound levels in the chambers were < 66 dB SPL. General Assessment Animals were observed for mortality (and presumably clinical signs) and body weights were measured weekly during the exposure and recovery periods. Organ weights were not evaluated. Surgical Procedures Electrodes consisting of three silver balls connected to a microconnector by silver wires were placed at points on the skulls of the anesthetized rats. The active electrode was placed at the lambda point over the inferior colliculus; the reference electrode was placed 2 mm posterior to the bregma and 6 mm to the right of the midline; the group electrode was placed over the nasal bone 2 mm anterior to the nasal suture and 2 mm lateral to the midline on the right. The apparatus was cemented to the skull with dental acrylic. Each rat was given 50 mg of ampicillin and allowed to recover from the effects of surgery for at least 7 days before the

	electrophysiological recordings were started. Neurophysiological Evaluation The interval between daily exposure and the electrophysiological measurements was 16 hours, except at weekends when the interval was 40 hours. Electrophysiological measurements were made at the end of the 4^{th}, 8^{th}, and 13^{th} weeks of exposure and at the end of the 8^{th} week of recovery (week 21). Neurophysiological measurement with a Racia computerized system was performed at a room temperature of 23 ± 1ºC. During the brainstem auditory-evoked response (BAER) recordings, the test animal was held in a restraining device and housed in an acoustically shielded chamber (ambient temperature of the chamber was 23 ºC). Fifty measured clicks (100 dB SPL) were used to elicit BAER. The BAER responses were recorded with a bandpass of 110 Hz-3.2 kHz. The averaged response was recorded after 1,024 clicks. The stimulus rate was 10/clicks presented in 5-dB steps. The evoked activity was analyzed for 10 ms following each click. An artifact rejection system automatically suspended BAER averaging during movement. Before and after the recording sessions, the animals' core temperatures were monitored by a rectal probe. Each averaged response was plotted. Audiometric thresholds were estimated at four frequencies (2,4,8, and 16 kHz) by visual inspection of the auditory brainstem responses as the lowest level of intensity were component N_vP_5 reached an amplitude of 1 µV. Morphological Evaluation Following the 8^{th} week of the recovery period, 8 rats/group were anesthetized and perfused via the open left ventricle with phosphate-buffered solution of 4% paraformaldehyde then 4% glutaraldehyde. The temporal bones were removed from the skull, the typmanic bullae were opened and the cochlea exposed. After perforating the apex and the round and oval windows, the cochleae were perfused with 1% osmium tetroxide in phosphate buffer (ph 7.4) then dehydrated in 30-70% ethanol. The bony capsule and membranes were removed and the organ of Corti and basilar membranes were dissected from the rest of the cochlea under a dissecting microscope. The surface preparations were mounted in glycerin/phsosphate buffer on glass slides with glass coverslips and observed with a Leitz light microscope. Cytocochleograms (total cell count) were constructed from the surface preparation, with the frequency coordinates of the organ of Corti (Muller, *Hear Res*, 49, 331-340, 1990). Four left and four right cochleas from eight rats were processed and counted in each group, including controls.
Results:	LOAEL (Concentration producing OHC loss) – 200 ppm *Calculated Values*: OHC EC50 = 371 ppm TLAEL calculated from the mean losses in the controls + 1.96 SD = 114 ppm TLAEL calculated from the mean losses in the controls + 2.58 SD =

	120 ppm TLAEL calculated from the mean losses in the controls + 3.29 SD = 130 ppm
Remarks:	Chamber Concentrations Mean measured inhalation exposure concentrations of ethylbenzene were 199, 404, 599 and 803 ppm. *All results are presented by target inhalation level.* Survival and Body Weight In the 800 ppm group, one rat lost its head plug during a recording session at the 14th week of the experiment, another rat died of unknown causes and one rat had to be sacrificed due to the development of a neck tumor. There was no significant difference in weight gain between the controls and the groups exposed to ethylbenzene. Neurophysiological Evaluation The audiometric thresholds of the animals exposed to 400, 600 and 800 ppm ethylbenzene were higher than controls at the 4 frequencies studied (note: results were provided in figures, hence specific group data values are not presented). These threshold shifts appeared from the 4th week of exposure onwards. The highest hearing losses were observed in the groups exposed to 600 and 800 ppm. They ranged from 44 dB at 2kHz to 49 dB at 16 kHz. They did not increase significantly throughout the exposure period. No recovery was observed 8 weeks after the end of exposure., when the losses were 43 dB at 2 and 4 kHz, 49 dB at 8 kZ and 53 dB at 16 kHz. The hearing losses were smaller in the 400 ppm exposed groups, ranging from 23 to 27dB depending on the frequencies studied at the end of the recovery period. No shift in audiometric thresholds was observed in the controls and in the group exposed to 200 ppm. Morphological Examination There was no significant hair cell loss in the controls. Exposure to 800 and 600 ppm caused nearly complete hair cell loss in the three rows of the OHC of the organ of Corti. Only the basal part of the cochlea, which transcribes the high frequencies, was partly spared. There were also inner hair cell (IHC) losses in the basal part of the organ of Corti. Quantitative evaluation of the IHC losses indicated that, on average, they reached 32 and 14% in the 800 and 600 ppm ethylbenzene exposed groups, respectively. Exposure to 400 ppm ethylbenzene caused considerable OHC losses, mainly in the apical and the upper and lower medium parts of the organ of Corti. The highest losses occurred in the third row and the lowest in the first row. Occcasional IHC losses were observed in the basal part of the organ of Corti. Exposure to 200 ppm caused significant losses (up to 30% losses in the mid frequency range) in the third row of the OHC in four of the eight animals. The average losses in the third row of the OHC of the animals exposed to 200 ppm were 4% and the EC50 was found to be 371 ppm. The theoretical lowest adverse effect levels (TLAELs) calculated from the three statistical upper confidence limits, i.e. 95, 99 and 99.9%, were 114, 120, and

	130 ppm, respectively.
Conclusion:	Ethylbenzene produced moderate to severe ototoxicity in young adult rats exposed for 4 to 13 weeks to concentrations ranging from 200 to 800 ppm. Repeated exposures of rats to ethylbenzene at concentrations of 400 ppm and greater produced significantly higher audiometric thresholds that did not recover 8 weeks after exposure ceased. Following the 8 week recovery period, Outer Hair Cell losses were present with increasing severity (4% to nearly 100%, respectively) in the rats at 200 to 800 ppm ethylbenzene.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Gagnaire, F., Langlais, C., Grossmann, S. and Wild, P. (2006). Ototoxicity in rats exposed to ethylbenzene and to two technical xylene vapours for 13 weeks. *Arch. Toxicol.* (electronic publication)

[RS - 35]

Developmental Neurotoxicity

Test Substance:	Ethylbenzene
CAS RN:	100-41-4
Purity:	99.9%
Method:	EPA Health Effects Testing Guidelines OPPTS 870.6300 and OECD Revised Draft Guideline 426 – Developmental Neurotoxicity Study
Type:	Developmental Neurotoxicity Study
GLP:	Yes
Year:	2004
Species/Strain:	Crl:CD® (Sprague-Dawley) IGS BR
Sex:	F_1: Females F_2: Males and Females
#/sex/dose:	F_1: 25 Females/group F_2: 40 Pups/sex/group
Vehicle:	Air (Inhalation) Corn Oil (Gavage)
Route of Administration:	Inhalation Vapor (Groups 1-4) Oral Gavage (Group 1-4 females; lactation days 1-4)
Exposure Period and Frequency of Treatment:	**F_1**: Inhalation vapor - F_1 females potentially exposed *in utero* and through nursing during lactation; daily 6-hour exposures after weaning; females exposed for a minimum of 70 consecutive days prior to mating, through gestation day 20 and from lactation day 5 through the day prior to euthanasia Oral gavage (females that delivered only) - Daily doses for 4 consecutive days (lactation days 1-4) **F_2**: No direct exposure - F_2 offspring potentially exposed *in utero* and through nursing during lactation
Doses/Concentrations:	Inhalation vapor: 25, 100 and 500 ppm (parts per million) Ethylbenzene Oral gavage: 26, 90 and 342 mg/kg bwt/day (8.67, 30 and 114 mg/kg bwt, respectively, administered three times per day at approximately 2-hour intervals) at 1 mL/kg bwt/dose
Control Group:	Air (Inhalation)

	Corn Oil (Gavage)
Statistical Methods:	Parametric one-way analysis of variance (ANOVA): functional observational battery data Parametric one-way analysis of variance (ANOVA) for normal and homogeneous data or Kruskal-Wallis nonparametric ANOVA for non-homogeneous or non-normal data: brain morphometry data Parametric one-way analysis of variance (ANOVA): functional observational battery data, Biel maze data (straight channel and overall mean time to escape and overall mean errors, and mean probe time and probe errors), ambulation counts measured in the locomotor activity assessment and average response measured in the acoustic startle assessment Univariate repeated measures ANOVA (RANOVA): intrasession total counts measured in the locomotor activity assessment and intrasession peak response and latency to peak response measured in the acoustic startle assessment Two-tailed Fisher's Exact test: functional observational battery data which yielded scalar and descriptive data and histopathological findings
Remarks for Test Conditions:	<u>Animals and Maintenance</u> - Species and strain: rat, Crl:CD (SD)IGS BR - Housing: individually housed in suspended wire mesh cages - Diet: Certified Rodent Lab Diet 5002 (PMI Nutrition International Inc.), *ad libitum*, except during exposure - Water: reverse osmosis-treated tap water, *ad libitum* except during exposure - Environment: controlled to 71 ± 5 ºF, 50 ± 20% relative humidity, 12 hour light/dark cycle, 10 air changes/hour <u>Chamber Conditions</u> Female F_1 were exposed in 2 m^3 stainless steel and glass whole-body inhalation chambers. Concentrations of ethylbenzene in the inhalation chambers were measured at least 10 times per exposure day by an automatic sampling system coupled to a gas chromatograph. <u>Treatment</u> Four groups of F_1 female Crl:CD®(SD)IGS BR rats (25/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, for 6 hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 0, 25, 100 and 500 ppm. F_1 females began inhalation exposure at weaning (postnatal day 22). Inhalation exposure of the F_1 females continued throughout mating and gestation through gestation day 20. Inhalation exposure of the F_1 females was suspended from gestation day 21 through lactation day 4. On lactation days 1 through 4, the F_1 females received the vehicle, corn oil, or test article in the vehicle via oral gavage at dose levels of 0, 26, 90 and 342 mg/kg bwt/day (divided into three equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg/dose. Inhalation exposure of the F_1 females was re-initiated on lactation

	day 5 and continued through the day prior to euthanasia. For reporting purposes, F_2 group designations were 0 ppm, 25 ppm, 100 ppm and 500 ppm. Inhalation/gavage exposure group designations for F_1 females were 0 ppm/0 mg/kg bwt/day, 25 ppm/26 mg/kg bwt/day, 100 ppm/90 mg/kg bwt/day and 500 ppm/342 mg/kg bwt/day. Preparation of Dosing Solutions Oral dosing solutions were prepared weekly in corn oil vehicle and stored at room temperature for a period that did not exceed 10 days in duration. Analysis of Dosing Solutions An aliquot from each formulation was taken from each weekly preparation and analyzed by GC. Stability was determined over 10 days (room temperature). Assessments A total of 40 pups/sex/group (2 pups/sex/litter, if possible) from the F_2 generation were selected for assessment of developmental neurotoxicity. In addition to the standard assessments included in the 2-generation component of the study, functional observational battery (FOB) evaluations were performed for F_1 females on gestation days 6 and 12 and lactation days 10 and 21. In addition to the standard litter and developmental landmark assessments included in the 2-generation component of the study, neurobehavioral evaluations were conducted on 2 subsets (each of 20/sex/group) of F_2 offspring (Subset A: FOB evaluations on postnatal days 4, 11, 22, 45 and 60, locomotor activity evaluations on postnatal days 13, 17, 21 and 61, acoustic startle response evaluations on postnatal days 20 and 60, and learning and memory evaluations in a Biel water maze task initiated on postnatal day 62; Subset B: learning and memory evaluations in a Biel water maze task beginning on postnatal day 26). Following *in situ* perfusion, brain weights and brain dimensions (length and width) were measured for 10 F_2 pups/sex/group on postnatal day 21 (Subset C) and on postnatal day 72 (a portion of animals from Subset A). In addition, a microscopic examination was conducted of the brains (postnatal day 21) or representative portions of the central and peripheral nervous systems (postnatal day 72), including brain morphometric evaluation, of 10 F_2 rats/sex/group from the control and high-exposure groups. F_2 rats used for neurobehavioral testing that were not selected for neuropathology and brain dimension measurements were necropsied on either postnatal day 33 (Subset B) or postnatal day 72 (Subset A).
Results:	Parental Toxicity NOAEL – 500 ppm or 500 ppm/342 mg/kg bwt/day, NOEL – 100 ppm or 100 ppm/90 mg/kg bwt/day Developmental Neurotoxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt/day
Remarks:	Chamber Concentrations Mean measured inhalation exposure concentrations were 0, 25, 101 and 500 ppm for the F_1 females. *All results are presented by target inhalation or dosage level.*

	Survival, Clinical Signs, and Body Weight There were no adverse test article-related effects on survival, clinical findings, or body weight in the F_1 generation dams and F_2 offspring. General Pathology No macroscopic findings were noted at any exposure level in the F_1 generation dams and F_2 offspring. Organ weights and microscopic pathology were unaffected in F_2 offspring. Neurologic and Neurodevelopment Evaluation *F_1 FOB*: No remarkable differences were apparent between the control and test-article exposed groups on maternal FOB evaluations were conducted on gestation days 6 and 12 and lactation days 10 and 21. None of the differences from the control group were statistically significant. *F_2 FOB*: No remarkable differences were apparent between the control and test-article exposed groups (Subset A) on FOB evaluations were conducted on postnatal days 4, 11, 22, 45 and 60. No statistically significant differences from the control group were noted. *F_2 Locomotor Activity*: No ethylbenzene-related effects (Subset A animals) on motor activity counts were observed. There were no statistically significant differences among the groups in activity parameters during the preweaning period (postnatal days 13, 17 and 21). However, the overall pattern of total session activity counts across the testing days during preweaning period did appear to suggest an acceleration in the standard developmental activity pattern for offspring from parentally-exposed rats, as indicated by somewhat higher counters on postnatal days 13 and 17 relative to the concurrent controls. This apparent alteration was observed as the result of an unusual within-session pattern of activity, normally considered habituation, in the control groups on postnatal days 13 and 17, ages prior to the normal development of habituation in the rat. Therefore, the apparent differences in the overall preweaning activity pattern of activity were attributed to an abnormal activity pattern in the control animals and not to parental exposure to ethylbenzene. There was a main effect on treatment ($p<0.05$) on mean total activity for females on postnatal day 61. A post-hoc Dunnett's test determined that activity for the 25 ppm group females was significantly increased ($p<0.05$) compared to the control group. Similar increases in total activity were not observed in the 100 and 500 ppm group F_2 females. Therefore, the 21.6% increase in mean total activity observed in the 25 ppm group females on postnatal day 61 was not attributed to parental ethylbenzene exposure. No other statistically significant differences from the control group were noted in either sex on postnatal day 61.

	Total Motor Activity Counts – Females – Postnatal day 61 (0, 25, 100 ,500 ppm) 1988, 2417*, 1946, 2001 (* significantly different from the control group at p<0.05 using Dunnett's test, following repeated measures analysis) *Acoustic Startle Test*: No exposure-related trends were apparent in the 25, 100 and 500 pm groups F_2 males and females on performance measured in the acoustic startle test: peak amplitude [V_{max}], latency to maximum response amplitude [T_{max}], and average response amplitude [V_{ave}]. A statistically significant (p<0.05) main effect of treatment was observed in the F_2 males on postnatal day 60 with all groups exhibiting lower mean V_{max} values (37-49% lower than the control group). The differences in the 25 and 500 ppm group males were statistically significant (p<0.05) in spite of the high variability noted in all groups tested at this age. There were no statistically significant effects obtained in startle parameters measured on postnatal day 60 in the F_2 females, although mean peak response amplitude in animals from the high exposure group was decreased approximately 34% compared to the controls. Mean peak response amplitude values for males from the control group fell in the upper quartile of the WIL historical control range at this age, unlike the very low values obtained in these same animals at postnatal day 20. Also in the males, there was no indication of a dose-response relationship in startle amplitude, as a similar magnitude of response was obtained in all three ethylbenzene-derived groups. Additionally, there were no indications of decreased reactivity, arousal, sensory or motor deficits in relevant components of the FOB conducted on these same males also on postnatal day 60. If, indeed, the apparent decreases in peak startle amplitude in all male exposure groups were related to parental ethylbenzene exposure, some corroborative evidence of alterations in reactivity in the FOB would likely have been apparent, at least in males from the 500 ppm group. Therefore, the differences noted in males at this age were attributed to unusual control values and were not considered to be related to parental ethylbenzene exposure. V_{max} (millivolts) – ALL Trials - Males – Postnatal day 60 (0, 25, 100 ,500 ppm) 195.1, 108.4*, 122.3, 99.6* (* significantly different from the control group at p<0.05 using Dunnett's test, following repeated measures analysis) *Biel Maze Swimming Trials*: There were no biologically meaningful differences in swimming ability on day 1 or for the times to criterion (mean time to locate the submerged platform) during the learning and memory trials between the F_2 male and female pups in the ethylbenzene-exposed groups when compared to the respective control groups on postnatal day 26 (Subset B animals) or on postnatal day 62 (Subset A animals). The mean numbers of errors committed during the various phases of evaluation were similar among the respective test and control groups. Overall Biel maze performance and overall probe values in the exposure-derived groups were not significantly different for those values obtained in the respective control groups.

	Brain Weights and Measurements: No remarkable differences were apparent between the control and test article-derived groups when brain weights and measurements were compared. No statistically significant differences from the control group were noted. *Brain Morphometry:* No brain histomorphologic changes were noted in the postnatal day 72 offspring from the 500 ppm/342 mg/kg bwt/day group for measurements taken at Level 1 (height of the hemisphere and vertical thickness of the cortex), Level 3 (radial thickness of the cortex, vertical height between hippocampal pyramidal neuron layers, vertical height of the dentate hilus, and length of the ventral limb of the dentate hilus) or Level 5 (vertical thickness of the pons and base of lobule 9). No statistically significant differences from the control group were noted.
Conclusion:	Ethylbenzene at an exposure level of 500 ppm/342 mg/kg bwt/day did not adversely affect neurodevelopment in rats.
Data Quality:	1 – Reliable without restrictions. Study well documented and meets generally accepted scientific principles. No circumstances occurred that would have affected the quality or integrity of the data.
Reference:	Stump, D.G. (2003). An inhalation two-generation reproductive toxicity study of ethylbenzene in rats including developmental neurotoxicity assessment of the F_2 generation. Study Number – WIL-186030, WIL Research Laboratories, Inc., Ashland, OH. Sponsored by the Ethylbenzene Panel, American Chemistry Council, Arlington, VA.