# Appendix A

## Voluntary Children's Chemical Evaluation Program (VCCEP)
## Peer Consultations on Ethylbenzene
## February 22-23, 2007

## List of Attendees

## List of Attendees

Dr. Marcy I. Banton
Lyondell Chemical Company

Ms. Lynn L. Bergeson
Bergeson & Campbell, P.C.

Dr. James S. Bus
The Dow Chemical Company

Mr. David Hunt
The Dow Chemical Company

Dr. Ratpan Flora
NOVA Chemicals, Inc.

Dr. Michael L. Gargas
The Sapphire Group, Inc.

Dr. Sophie Jia
Chevron Phillips Chemical

Dr. Janet E. Kester
NewFields LLC

*Dr. Lawrence K. Low
ExxonMobil Biomedical Sciences, Inc.

Dr. Elizabeth Moran
American Chemistry Council

Dr. Ines Pagan
US Environmental Protection Agency

Dr. Linda G. Roberts
Chevron Energy Technology Company

Dr. Chad B. Sandusky
Physicians Committee for Responsible Medicine (PCRM)

Mr. Richard B. Stalzer
Ferro Corporation

Dr. Lisa M. Sweeney
The Sapphire Group

Mr. Jeffrey P. Viola
INEOS Styrenics

Dr. George Cruzan
ToxWorks (for SIRC)

*Web cast Participant

# Appendix B

## Voluntary Children's Chemical Evaluation Program (VCCEP)
## Peer Consultations on Ethylbenzene
## February 22-23, 2007

## Agenda, Overview, Panel Charge, Panelist Biographical Sketches and Conflict of Interest/Bias Disclosures, and Presenter Biographical Sketches

# Agenda

## *VCCEP Peer Consultation for Ethyl Benzene*

## Northern Kentucky University, METS Center

## February 22-23, 2007

# Thursday, February 22, 2007

**8:00 Registration and Check In**

**8:30 Meeting Convenes***

Welcome: Ms. Jacqueline Patterson, *TERA*
Introductions and Disclosures, Panel
Meeting Process: Dr. Michael Dourson, Panel Chair

**9:00 Sponsor Introduction**
Presenter: Dr. Elizabeth Moran, American Chemistry Council Ethylbenzene Panel

**Sponsor Presentation on Exposure Assessment**
Presenter: Dr. Janet Kester, NewFields, LLC
Clarifying Questions from Panel

**Public Comments on Exposure Assessment**
Clarifying Questions from Panel and Sponsors

**Panel Discussion of Exposure Assessment**
Discussion of Panel Charge Questions Regarding Exposure Assessment

**12:15 Lunch**

**1:15 Sponsor Presentation on Hazard Assessment**
Presenter: Dr. Marcy Banton, Lyondell Chemical Company
Dr. James Bus, The Dow Chemical Company
Clarifying Questions from Panel

**Public Comments on Hazard Assessment**
Clarifying Questions from Panel and Sponsors

**Panel Discussion of Hazard Assessment**
Discussion of Panel Charge Questions Regarding Exposure Assessment

**5:00 Adjourn**

* Chair will call mid morning and mid afternoon breaks at convenient times

# Friday, February 23, 2007

**8:00 Registration**

**8:30 Meeting Re-convenes***

**Sponsor Presentation on Risk Characterization**
Presenter: Dr. Michael Gargas, The Sapphire Group
Dr. Lisa Sweeney, The Sapphire Group
Clarifying Questions from Panel

**Public Comments on Risk Characterization**
Clarifying Questions from Panel and Sponsors

**Panel Discussion on Risk Characterization**
Discussion of Panel Charge Questions Regarding Risk Characterization

**12:15 Lunch**

**1:15 Sponsor Presentation on Data Needs**
Presenter: Dr. Elizabeth Moran, ACC Ethylbenzene Panel
Clarifying Questions from Panel

**Public Comments on Data Needs**
Clarifying Questions from Panel and Sponsors

**Panel Discussion on Data Needs**
Discussion of Panel Charge Questions Regarding Data Needs

**4:30 Closing Remarks and Evaluation of Meeting**

**5:00 Adjourn**

* Chair will call mid morning and mid afternoon breaks at convenient times

# Overview of the Peer Consultation Process

This document provides background information on the VCCEP pilot program and the peer consultation. It is presented in two parts: General Background on VCCEP and Overview of How *TERA* Organizes and Conducts VCCEP Peer Consultation Meetings. The expectations for panelists and their responsibilities before, during, and after the panel meeting also are briefly discussed. Please contact Dr. Dan Briggs at briggs@tera.org if you have questions or desire additional information.

## General Background on VCCEP

In the December 26, 2000, Federal Register, http://www.epa.gov/fedrgstr/EPA-TOX/2000/December/Day-26/t32767.htm, EPA announced the Voluntary Children's Chemical Evaluation Program (VCCEP) pilot program. This program is intended to provide data to enable the public to understand the potential health risks to children associated with certain chemical exposures. The key questions of the program are whether the existing data on a given chemical are sufficient to adequately characterize the potential hazards, exposures, and risks to children and prospective parents, and, if not, what additional data are necessary.

The VCCEP pilot program uses a tiered testing approach. For toxicity (health effects) data, specific types of studies have been assigned to one of three tiers. For exposure data, the types of studies required are less specific, but the depth of exposure information increases with each tier.

EPA asked companies which manufacture and/or import 23 chemicals found in human tissues and the environment to volunteer to sponsor an evaluation of their chemicals in a pilot of the VCCEP. Sponsorship requires the companies to collect or develop health effects and exposure information on their chemicals and then to integrate that information in a risk assessment and a data needs assessment. If data needs are identified through this process, the sponsor will choose whether or not to volunteer for any additional data generation or testing and whether to provide additional assessments. Thirty-five companies and ten consortia responded and volunteered to sponsor 20 chemicals in Tier 1.

*TERA* was awarded a Cooperative Agreement by EPA to design, develop, and manage a peer consultation process that would serve as a public scientific forum. One of the activities undertaken by *TERA* under this agreement is the VCCEP plot program. *TERA's* primary role in this program is to ensure it is a rigorous, science-based process for reviewing VCCEP assessments. Stakeholders should recognize the process as impartial and of significant technical merit and value. *TERA's* role in managing the peer consultation is undertaken primarily at the request of and for the benefit of non-federal VCCEP stakeholders, particularly the sponsors of VCCEP chemicals.

## Overview of How *TERA* Organizes and Conducts VCCEP Peer Consultation Meetings

*TERA* is an independent non-profit organization with a mission to protect public health through the best use of toxicity and exposure information in the development of human health risk assessments. For the VCCEP pilot program, *TERA*'s responsibilities include identifying and recruiting scientists with relevant expertise to comprise a peer consultation panel, identifying and managing conflict of interest and bias issues of the panel candidates, organizing and conducting the peer consultation panel meetings, and drafting and finalizing the meeting reports.

The panel meeting provides a science-based peer consultation on the data needs for the chemical, utilizing not only the assessment submitted by the sponsor, but also the expertise and knowledge of the panel. Members of the peer consultation panels are selected by *TERA* based on their expertise in scientific disciplines relevant to the chemicals, test methodologies, and risk assessment issues that will be discussed. Nominations for panel members are welcomed from all interested parties. *TERA* selects the panel members from among those nominated and also from among other qualified experts whom *TERA* independently identifies.

Each panel candidate discloses information regarding potential conflicts of interest and biases. *TERA* evaluates these disclosures in selecting the panel members following procedures in accordance with the U.S. Office of Management and Budget, the National Academy of Sciences, and the U.S. EPA. These procedures are described in more detail at http://www.tera.org/peer/COI.html.

Panel members also are selected to bring a wide range of views and perspectives to the peer consultations, reflecting the interest in VCCEP by a wide range of stakeholders. The panel does not attempt to reach consensus positions; rather, the individual opinions of each of the members are noted.

Members of the public are invited to attend the peer consultation meetings, and they are invited to provide brief oral and written technical comments on the assessment document for the panel's consideration. Recent panel meetings have been made available to pre-registered, off-site observers via real-time web casts.

*TERA* reviews the sponsor's VCCEP chemical assessment document and develops a panel charge to guide the panel in its discussions during the meeting. The panel charge focuses the meeting discussions by presenting specific items for the panel to address. General questions regarding completeness and interpretation of data are included, as well as more specific questions relevant to the hazard, exposure, or risk characterization of the specific VCCEP chemical being evaluated. The charge includes questions regarding data gaps and data needs and asks panelists to identify data needs and their rationale for them.

*TERA* is responsible for all meeting preparations including travel and logistics, announcements, distribution of the review materials, and assisting the panel. VCCEP peer consultation meetings generally follow a standard *TERA* process, beginning with a close examination of the sponsor's report and supporting documentation by the panel prior to the meeting.

At the beginning of the meeting, panelist disclosures regarding potential conflict of interest and bias issues are presented and discussed. *TERA* believes transparency in these matters is important and therefore discusses these openly at the meeting, allowing panel members to question one another. These disclosures are also part of the public record through inclusion in the meeting report. The Chair then discusses the ground rules for the meeting. Ground rules generally include the following items:

- Chair will call upon panel members in turn and will interrupt discussion if he thinks the topic is drifting. He will not call upon observers. Observers can talk to the Chair or to *TERA* staff during a break in the meeting if they wish to schedule a time to comment.

- If a panelist states a part of the assessment unacceptable, he or she will be asked to explicitly state what additional work would be needed to make it acceptable. The Chair may ask the panelist to work with the sponsor to resolve the issues during the breaks.

- Panel members will have provided premeeting comments before the meeting. These comments are informal and not part of the meeting record. They are initial thoughts that were shared with the sponsor and other panel members to help identify issues and new data. Panel members must raise items in their premeeting comments during the meeting in order for them to be included in the meeting record.

The meeting discussions are limited to panel members. One or two authors or sponsor representatives sit at the table to answer panel questions. These representatives are allowed to ask the panel members clarifying questions as needed. In order to avoid the appearance of undue influence on the panel, all parties are asked to refrain from discussing issues related to this review with panel members prior to the meeting or during the breaks unless a panel member initiates the discussion. Panel members are asked to summarize any substantive conversations for the rest of the panel and audience when the meeting reconvenes after the break.

The discussion period begins with the authors or sponsors making short presentations summarizing their report and possibly also addressing issues raised by the panelists in their premeeting comments. These presentations highlight salient issues and give the panel the opportunity to ask clarifying questions. The Chair then leads the panel in discussions, using the items in the panel charge. Individual panelists will be asked to share their opinions and defend them with scientific data and analysis.

*TERA* scientists take notes of the meeting discussions and prepare a draft meeting report summarizing the panelists' discussions, conclusions and recommendations. This report is not a transcript of the meeting but a summary of the key discussions and issues. Panel members are listed, but their individual comments are not attributed to them by name. The draft report is reviewed by the panel. The sponsors also are allowed to review the draft report, but they must limit their comments to matters of clarity and completeness regarding their presentations and statements made at the meeting. The meeting report includes copies of the sponsor presentation slides, a list of attendees, panel biographical sketches and COI/bias disclosures, and public comments. When finalized, the meeting reports are made available to the public on *TERA*'s Peer Review and Consultation website (http://www.tera.org/peer/welcome.htm).

# VCCEP Peer Consultation Panel Charge for Ethylbenzene

## Introduction

The primary objective of this Peer Consultation Panel is to discuss whether the potential hazards, exposures, and risks for children have been adequately characterized for ethyl benzene, based on the information contained in assessment documents submitted by the sponsors and on other available information. If the potential hazards, exposures, and risks cannot be adequately characterized, then data needs should be identified. The focus of the panel meeting is not on reviewing the adequacy of the report per se, rather a review of the adequacy of the available data. The panelists use the document and its references as a source of information, along with personal information and knowledge. The panel is not required to reach consensus positions on any issues or conclusions. Panelists who believe a chemical has not been adequately characterized will be asked to identify what additional information is needed and why they believe it is necessary. All the panelists will be encouraged to discuss and debate each other's suggestions and comments, providing scientific rationales for their points of view. *TERA* will compile the panel discussions in a meeting report that will be sent to the sponsor and made available to the public.

*TERA* has prepared this charge to help the panel discuss the sponsor's submission and address whether a chemical has been adequately characterized. The topics are consistent with the directions for VCCEP submissions given in the December 26, 2000, Federal Register: http://www.epa.gov/chemrtk/vccep/.

Panelists should keep in mind the following directives from the Federal Register regarding any recommendations for additional testing: (1) if specific toxicity studies are indicated, they should be chosen from the next tier of studies within the overall framework. They should allow flexibility to pursue either additional toxicity testing and/or exposure evaluation, allowing sponsors to select the option which will most quickly, directly, and cost-effectively reduce uncertainty and allow the creation of a risk assessment; (2) EPA is committed to avoiding duplicative testing, and to reducing, refining, and replacing animal testing when valid alternatives exist; (3) if relevant alternative test methods become validated, EPA will consider their immediate implementation in the program; (4) EPA encourages sponsors to combine tests where possible to conserve resources and reduce the number of animals required for testing; and (5) the Tier 2 and Tier 3 testing will be limited to chemicals for which there is a clear testing need.

**Exposure Assessment**

1. Discuss whether the fate of ethyl benzene is adequately understood, both in the environment and within the human body.

2. Are the potential sources of ethyl benzene exposure adequately identified? Are there other sources that should have been considered?

3. Discuss whether the available data are adequate regarding the following exposure aspects: sources, routes, frequency, duration, and intensity.

4. Discuss whether the data, exposure scenarios, age groupings, parameters, and assumptions used in the exposure assessment were appropriate to characterize risk to children. Should other data or scenarios have been evaluated or different assumptions used?

5. Discuss whether the exposure data are sufficient to assess subpopulations, such as a) the prospective parents, b) the embryo and fetus, c) the nursing infant, and d) the post-nursing child through adolescence to the age of sexual maturation.

6. Discuss whether the estimates of exposure are defensible and have been calculated correctly.

7. Discuss any other significant issues related to the ethylbenzene exposure assessment.

**Hazard Assessment**

8. Discuss whether the available information on local and systemic toxicity, acute and chronic toxicity, and ADME (absorption, distribution, metabolism, and elimination) is adequate to identify and assess potential hazards.

9. Discuss whether the hazard data are sufficient to characterize risk for subpopulations, such as a) the prospective parents, b) the embryo and fetus, c) the nursing infant, and d) the post-nursing child through adolescence to the age of sexual maturation.

10. Discuss any other significant issues related to the ethylbenzene hazard assessment.

**Risk Characterization**

11. The authors propose an updated reference dose (RfD) and reference concentration (RfC) that are different from what EPA has on its Integrated Risk Information System (IRIS). Discuss whether the noncancer toxicity benchmarks that were developed and used to characterize the adverse health effects of ethylbenzene (RfC and RfD) are scientifically defensible and appropriate to use for this risk characterization.

12. In discussing ethylbenzene's carcinogenicity results in animals, the authors present possible cancer modes of action for each tissue site that showed increased tumor incidence: exacerbation of chronic progressive nephropathy in the rat kidney, increased regenerative cell proliferation caused by reactive metabolites in the mouse lung, and phenobarbital-like induction in the mouse liver. The authors conclude that these cancer modes of action, which occur in rats and mice, cannot be extrapolated to humans, and therefore they are not relevant for assessing human risk.

    a. Discuss whether the modes of action for carcinogenicity suggested by the authors for the animal tumors are scientifically defensible.

    b. Discuss whether the modes of action for carcinogenicity occurring in the test animals are relevant for human risk assessment.

13. The authors prepared a cancer dose-response assessment for ethylbenzene following EPA's Guidelines for Carcinogenic Risk Assessment (EPA, 2005). The assessment included the use of internal dose measures as determined by a physiologically-based pharmacokinetic (PBPK) model. Discuss whether the method used and the conclusion drawn from the ethylbenzene cancer dose-response assessment is scientifically defensible.

14. Discuss whether the PBPK modeling data presented in the report (Appendices P through S) adequately support the existence of biologically meaningful differences in ethylbenzene metabolism between species (e.g., between mouse and human lung tissue).

15. Discuss whether the risk characterization adequately characterized the risk to subpopulations, such as a) the prospective parents, b) the embryo and fetus, c) the nursing infant, and d) the post-nursing child through adolescence to the age of sexual maturation.

16. Discuss any other significant issues related to the ethylbenzene risk characterization.

**Data Needs**

17. Identify any additional exposure data or analyses that are needed and discuss why this information is necessary for the next VCCEP tier. Differentiate between *data gaps* and *data needs*.

18. Identify any additional hazard information that is needed and discuss why it is necessary. Differentiate between *data gaps*[1] and *data needs*[2]. Focus on those studies indicated for the next VCCEP tier.

[1] In the context of the VCCEP pilot program, *data gaps* are defined as areas that could benefit from additional data, additional analyses, or clearer presentation.
[2] In the context of the VCCEP pilot program, *data needs* are defined as data gaps requiring additional work before the potential risk to children can be adequately characterized. Not all data gaps will be considered data needs. The panelists may consider the risk characterization results when determining whether a data gap is a data need.

# Biographical Sketches and Disclosure Statements

Following NAS guidance, *TERA* creates panels that have a balance of scientific viewpoints on the issues to be discussed. As a result, *TERA*'s panels have a broad and diverse range of knowledge, experience, and perspective, including diversity of scientific expertise and opinion. In addition, *TERA* creates panels with multiple organizational perspectives (e.g., academic, consulting, environmental, government, and industrial/commercial). However, panel members serve as *individuals*, representing their own personal scientific opinions. They do not serve as representatives of their companies, agencies, funding organizations, or other entities with which they are associated. Their opinions should not be construed to represent the opinions of their employers or those with whom they are affiliated.

*TERA* is conducting this VCCEP Ethylbenzene Peer Consultation under its Peer Consultation Program. This program is principally funded by a Cooperative Agreement with the U.S. Environmental Protection Agency (EPA), the purpose of which is to design, develop, and manage a peer consultation process that will serve as a public scientific forum. *TERA's* role in managing the peer consultation is undertaken primarily at the request of and for the benefit of non-federal stakeholders, particularly the sponsors of VCCEP chemicals.

*TERA* has performed work for organizations associated with VCCEP, both in the past and at the present time. These organizations include the EPA, the American Chemistry Council (ACC), and some companies whose parent organizations or subdivisions are sponsors of the ethylbenzene submission (BP, Dow Agrosciences, GE Aircraft). None of the work *TERA* did with these organizations was on ethylbenzene or regarding VCCEP. *TERA* has conducted assessments and analysis for a number of other chemicals included in the VCCEP pilot program in the past (i.e., acetone, decabromodiphenyl ether, methyl ethyl ketone, toluene, and xylenes) and currently is working on projects involving trichloroethylene. This work has been done for a variety of public and private sponsors, but none of it is directly related to the VCCEP assessments.

The purpose of this VCCEP Ethylbenzene Peer Consultation is to gather the scientific opinions of a range of experts with relevant knowledge and experience, including those who may be affiliated with organizations or companies with an interest in the outcome. All panelists were selected by *TERA* based upon their expertise and qualifications. They are employed by many types of organizations. *TERA* strived to create a balance of expertise and affiliations for this consultation meeting; however, *individual panel members represent their own expertise and views*, not those of their employer, of any group who may have nominated them, or any group with whom they may be associated. This panel is a distinguished group with many years experience in a wide range of disciplines.

An essential part of panel selection is the identification and disclosure of conflicts of interest and biases. Prior to selecting the core and *ad hoc* panelists, *TERA* requested each panel member to complete a questionnaire to determine whether their activities, financial holdings, or affiliations could pose a real or perceived conflict of interest or bias. The completed questionnaires were reviewed by *TERA* staff and discussed further with panel candidates as needed. (See http://www.tera.org/peer/COI.html for *TERA's* conflict of interest and bias policy and procedures for panelist selection).

*TERA* has determined, and each panel member has certified, that he or she has no conflicts of interest and is able to objectively participate in this peer consultation.

## Dr. Michael Dourson

Dr. Dourson directs Toxicology Excellence for Risk Assessment (*TERA*), a nonprofit corporation dedicated to the best use of toxicity data for estimating risk assessment values. *TERA*'s projects include the development of complex risk assessments, such as soluble nickel salts; research into improvements of risk methods, such as differential sensitivity of children and adults to chemical toxicity, organizing peer review and consultation meetings for risk assessment topics and documents; and education and outreach on risk assessment values through lectures and data bases, including the International Toxicity Estimates for Risk (*ITER*).

Before founding *TERA* in 1996, Dr. Dourson held leadership roles in the U.S. Environmental Protection Agency (EPA) for fifteen years; as chair of EPA's Reference Dose (RfD) Work Group, charter member of the EPA's Risk Assessment Forum and chief of the group that helped create the Integrated Risk Information System (IRIS) in 1986. Dr. Dourson received his Ph.D. in Toxicology from the University of Cincinnati and a B.A. in biology from Wittenberg University. Dr. Dourson's research interests include investigating methods to extrapolate toxicity data garnered on experimental animals or healthy adults to the appropriate sensitive human population. Topics such as adversity of effect and characterization of risk are also of interest.

Dr. Dourson has served on numerous expert panels, such as EPA's peer review panels for IRIS assessments and its Risk Assessment Forum, *TERA*'s International Toxicity Estimates for Risk (*ITER*) independent peer reviews and consultations, FDA's Science Board Subcommittee on Toxicology, the National Science Foundation's Health Advisory Board, and the Society of Toxicology's harmonization of cancer and non-cancer risk assessment. Dr. Dourson has also organized over 16 symposia for 9 different organizations on a variety of topics, including: risk communication; chromium; information resources for toxicology and environmental health; risk assessment of essential trace elements; risk characterization; EPA's IRIS; uncertainty in risk assessment techniques; statistical and dose response models in risk assessment; workshop on benchmark dose methodology; basics of risk assessment; improvements in quantitative noncancer risk assessment; and neurotoxicity risk assessment.

Dr. Dourson is a Diplomate of the American Board of Toxicology and served on its Board as President, Vice President, and Treasurer. He is the past Secretary for the Society for Risk Analysis, and has also served as presidents of the Dose-Response Specialty Group of the Society for Risk Analysis, of the Society of Toxicology's Specialty Section on Risk Assessment and of the Ohio Chapter of the Society for Risk Analysis. He is currently on the editorial board of three journals. Dr. Dourson has published more than 100 papers on risk assessment methods, has co-authored well over 100 government risk assessment documents, and has made over 100 invited presentations.

Dr. Dourson is a core panel member. He was selected for the core panel because of his expertise in toxicology, risk assessment, and derivation of non-cancer risk values.

### Disclosure

Dr. Dourson is Director of *TERA*. As an employee of *TERA*, Dr. Dourson has contributed in the past to research and development activities sponsored by ACC, EPA, and some of the Ethylbenzene VCCEP sponsor companies. In 1985, Dr. Dourson participated in EPA's RfD/RfC Work Group on ethylbenzene, and, in 2003, he reviewed EPA's Air Toxics Research Plan and Multiple Year Strategy documents, which may have included ethylbenzene. His employer, *TERA*, has done work for ACC and also for three firms whose parent companies or company subdivisions are sponsoring the ethylbenzene submission (BP, Dow Agrosciences, and GE Aircraft). None of this work by *TERA* was specifically on ethylbenzene.

*TERA* has determined that Dr Dourson has no conflicts of interest. His previous activities with ACC and EPA, and his employer's work with sponsors and contributing consultants are being disclosed to assure transparency. *TERA* does not believe these activities will impair Dr. Dourson's scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. G.A. Shakeel Ansari

Dr. Ansari is a Professor in the Departments of Biochemistry & Molecular Biology and Pathology at the University of Texas Medical Branch (UTMB), Galveston, TX. He also serves as the director of Biotransformation Research Core of National Institute of Environmental Health Sciences (NIEHS) center. Dr. Ansari is a member of Graduate School of Biomedical Sciences, NIEHS center, and Sealy Center for Environmental Health and Medicine (all at UTMB). His research focuses on the toxicity of environmental chemicals and their metabolites, lipid adducts resulting from non-oxidative metabolism of environmental chemicals, protein adducts of environmental chemicals as neoantigens, oxidative stress induced by environmental chemicals and analytical toxicology. He receives research support from the National Institutes of Health. Chemicals of interest are hydrocarbons, alcohols and amines. His teaching responsibility at UTMB includes both at the level of Medical School and Graduate School. Besides training graduate students and postdoctoral fellows, he teaches in various courses related to toxicology and/or metabolism.

Dr. Ansari received his Ph.D. degree in chemistry from Aligarh Muslim University, India. After obtaining post-doctoral training at University of Idaho and UTMB, he joined the faculty of UTMB in 1979 and achieved the rank of Professor in 1990.

Dr. Ansari has co-authored more than 125 peer-reviewed publications, dealing with molecular, biochemical and analytical toxicology. Several reviews, book chapters etc. were also written on related subjects. Over the years, his research was supported by various national and international agencies such as National Institutes of Health (NIH), National Institutes for Occupational Safety and Health (NIOSH), U.S. Environmental Protection Agency (EPA), and the World Health Organization (WHO).

Dr. Ansari has extensive experience in peer-reviewing drafts of various toxicology profiles for the Agency for Toxic Substances and Disease Registry (ATSDR), as well as risk assessment issue papers for the U.S. Environmental Protection Agency through Eastern Research Group, Inc. He also served as a charter member on an NIH study section dealing with peer-reviews of toxicology-related grants and Superfund grant applications, and he is an *ad hoc* reviewer for the National Science Foundation. Besides reviewing manuscripts for various journals, he serves as an Associate Editor of *Bulletin of Environmental Contamination and Toxicology*, and he is on the editorial aboard of *Toxicology and Applied Pharmacology* and the *Journal of Toxicology and Environmental Health, Part A.* He is an active member of several professional societies including the Society of Toxicology, the International Society for the Study of Xenobiotics, the Metabolomics Society, the American Chemical Society, and the American Society of Biochemistry and Molecular Biology.

Dr. Ansari is an *ad hoc* panel member. He was selected for the ethylbenzene panel because of his expertise in biochemical mechanisms of toxicity and biological markers of chemical exposure, and also because he served as a peer reviewer of the toxicological profile for ethylbenzene prepared by the ATSDR in 1999.

### Disclosure

Dr. Ansari received research support from the Chemical Manufacturers Association (CMA) (now known as the American Chemistry Council [ACC]) from 1994-1997 for work related to 2-butoxyethanol. This support was in the form of a grant from CMA for which Dr. Ansari was a co-investigator. The work did not involve ethylbenzene.

*TERA* has determined that Dr Ansari has no conflicts of interest. His previous research support from CMA is being disclosed to assure transparency. *TERA* does not believe this former support will impair Dr. Ansari's scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. Susan Borghoff

Dr. Borghoff is the Scientific Director of Investigative Toxicology at ILS, Inc. ILS is a service contract laboratory in Research Triangle Park, North Carolina, that works with both federal and commercial clients. Prior to this position (since April 2006), Dr. Borghoff was Senior Staff Scientist at CIIT Centers for Health Research in the Research Triangle Park. Her research interests have focused on understanding the mode-of-action by which specific chemicals cause kidney toxicity and cancer in rats with a view to understanding the relevance of this response for human risk assessment. She also is interested in understanding the metabolism and pharmacokinetics of various chemicals with emphasis on the development of physiologically based pharmacokinetic models that can be used for risk assessment. Dr. Borghoff has conducted research on the developmental pharmacokinetics of estrogen-like compounds such as genistein. Along with Dr. Borghoff's research program at CIIT, she was also the Director of Education Programs for two years, which involved oversight of the pre- to post- graduate training programs and K-12 educational outreach activities.

Dr. Borghoff received the Frank R. Blood Award in 1994 for the best paper of the year published in one of the Society of Toxicology research journals and a Society of Toxicology Risk Assessment Specialty Section Award in 2000. She is a member of the North Carolina Chapter of the Society of Toxicology and the National Society of Toxicology, in which she has served on the Program, Awards and Education Committees. She is currently an Associate Editor for *Toxicological Sciences* and on the editorial board for *Chemical Biological Interactions*. Dr. Borghoff has served as a reviewer on numerous panels and as a working group member for national and international organizations such as the U.S. Environmental Protection Agency (U.S. EPA), the National Cancer Institute, the International Programme on Chemical Safety, the European Centre for Ecotoxicology and Toxicology of Chemicals, and the International Agency for Cancer Research. She has also been a reviewer for the National Institute of Environmental Health Sciences (NIEHS) Superfund Basic Research Program Grant, research grants for the U.S.EPA on children's health issues, and most recently a reviewer on the NIEHS Special Emphasis Panel for Absorption, Distribution, Metabolism and Excretion (ADME) Chemical Disposition in Mammals.

Dr. Borghoff received her Ph.D. and MSPH in Environmental Sciences and Engineering from the University of North Carolina, and a B.S. in Chemistry from East Stroudsburg University in Pennsylvania. She is a Diplomate of the American Board of Toxicology.

Dr. Borghoff is an *ad hoc* panel member. She was selected for the ethylbenzene panel because of her expertise in using physiologically based pharmacokinetic (PBPK) models to assess human risk and in relating pharmacokinetics to renal toxicity and carcinogenicity.

### Disclosure

From 1987 to 2006, Dr. Borghoff was employed by CIIT Centers for Health Research. During that time, some of her research was funded by Lyondell Chemical Company (research not related to ethylbenzene). In 2006, Dr. Borghoff served as a consultant for Chevron and for the Lyondell Chemical Company; this consultant work (not related to ethylbenzene) is now completed. Dr. Borghoff's employer is currently doing work for the ACC and for the Lyondell Chemical Company. Dr. Borghoff is associated with this work, but it is not related to ethylbenzene. Dr. Borghoff currently is also a member of an expert panel providing oversight to a bioassay on a non-ethylbenzene chemical sponsored by a group of companies that includes Chevron.

*TERA* has determined that Dr. Borghoff has no conflicts of interest. Her previous sponsor-related research support and consulting activities, and her current activities are not related to ethylbenzene, but

they are being disclosed to assure transparency. *TERA* does not believe Dr. Borghoff's previous or current work will impair her scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. John Christopher

Dr. Christopher is a staff toxicologist with the Department of Toxic Substances Control (DTSC), California Environmental Protection Agency (Cal EPA). In this position he reviews, critiques, and approves assessments of risk to human health and ecological risk assessments at military facilities and other hazardous waste sites and permitted facilities in California. He constructs multi-pathway risk assessments to identify numerical criteria for classifying hazardous levels of metals and organic chemicals in waste. He also uses Monte Carlo methods in various exposure settings to identify levels protective of human health. He has received Certificates of Recognition for contributions resulting in the successful transfer of a hazardous waste landfill at a former naval shipyard in Vallejo, California, for a prescribed burn to uncover unexploded ordnance at a former fort in Monterey, California, and also for cleanup of a fleet industrial supply center in Alameda, California. In addition, he has received a Sustained Superior Accomplishment Award from California Department of Toxic Substances Control for risk assessment of metals in hazardous waste.

Prior to his current position with the State of California, Dr. Christopher conducted risk assessments for ICF Kaiser Engineers and IT Corporation. He also worked for research laboratories where he conducted and managed animal studies.

Dr. Christopher earned a B.S in Biology from Georgetown University, Washington D.C., and a M.A. in Pharmacology from Stanford University, Palo Alto, California. He received his Ph.D. in Biological Science from Oregon State University, Corvallis, Oregon.

Dr. Christopher is a Diplomate of the American Board of Toxicology and a former member of this Board. He has served as President and held several other offices in the Risk Assessment Specialty Section of the Society of Toxicology (SOT) and also in SOT's Northern California Chapter. He is a peer reviewer for *Toxicological Sciences, Risk Analysis*, *Human and Ecological Risk Assessment,* and *CRC Critical Reviews in Toxicology.*

Dr. Christopher is a core panel member. He was selected for the core panel because of his experience in toxicology, multi-pathway risk assessment, and the evaluation of general and site-specific exposure scenarios.

### Disclosure

Dr. Christopher's current responsibilities at Cal EPA include evaluating exposures from hazardous waste sites that may contain ethylbenzene. In his regulatory capacity, he requires authors of risk assessments submitted to DTSC to use the values for toxicity of ethylbenzene maintained by Cal EPA, and the Integrated Risk Information System (IRIS) maintained by the U.S. Environmental Protection Agency. Because of these job-related responsibilities, Dr. Christopher requested the following statement in this disclosure: "Dr. Christopher performs scientific peer consultation for *TERA* as a private individual. His employer, the California Department of Toxic Substances Control, is not bound in any way by the opinions he expresses or by consensus agreements to which he chooses to be a party." In 2002, Dr. Christopher evaluated research proposals in a paid capacity for the American Chemistry Council (ACC), but these proposals were not related to ethylbenzene or VCCEP.

*TERA* has determined that Dr Christopher has no conflicts of interest. His current responsibilities at Cal EPA and his past activities are being disclosed to assure transparency. *TERA* does not believe his current or past activities will impair Dr. Christopher's scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. John DeSesso

Dr. DeSesso is a charter member of the technical staff of Mitretek Systems, an independent, not-for-profit company that was formed from several parts of The MITRE Corporation. Dr. DeSesso is a Senior Fellow and the Director of the Biomedical Research Institute at Mitretek Systems. Dr. DeSesso has extensive experience in reproductive and developmental toxicity, risk assessment, ecological risk assessment, and the use of bioavailability in risk assessments.

Dr. DeSesso received his Ph.D. in Anatomy and Teratology from the Medical College of Virginia at Virginia Commonwealth University. He is a Diplomate of the American Board of Forensic Examiners and the American Board of Forensic Medicine, specializing in anatomy and risk assessment, and a Fellow of the Academy of Toxicological Sciences. Prior to joining Mitretek Systems, Dr. DeSesso was a Senior Principal Scientist at MITRE Corporation where he evaluated chronic studies (with special attention to reproductive toxicity and teratology) for the U.S. Environmental Protection Agency's (EPA) Office of Pesticides, conducted biostatistical analyses of data and risk assessment techniques, predicted toxic effects based upon structure-activity relationships for new chemicals, provided quality assurance of risk assessments performed by contractors for the U.S. Air Force, and performed independent research into the mechanisms that underlie chemically induced birth defects. Dr. DeSesso's research focus has been the elucidation of the mechanisms underlying teratogenesis and designing strategies to ameliorate the untoward effects.

Dr. DeSesso is currently a faculty member at Georgetown University School of Medicine, Rosalind Franklin University of Medicine and Science, San Diego State University Graduate School of Public Health, and the University of North Texas Health Sciences Center. He is an active member of numerous scientific societies where he has held various office positions, such as the Academy of Toxicological Sciences, the American College of Toxicology, the American Society for Reproductive Medicine, the Society for Risk Analysis, the Society of Toxicology, and the Teratology Society.

Dr. DeSesso has been an active member of the peer-review process reviewing manuscripts for major journals and grant proposals on a national and international level (e.g., EPA, United States-Israel Binational Science Foundation, National Institutes of Health, National Institute for Environmental Health Sciences [NIEHS]). He has been invited frequently to serve as the chairman of scientific sessions at national and international scientific meetings, especially those involving mechanisms or amelioration of developmental toxicity and ecological risk assessment. He has served as an invited faculty member or invited participant on many panels, refresher courses, and working groups that have been sponsored by a variety of federal agencies (e.g., EPA, U.S. Food and Drug Administration, NIEHS) and professional societies (e.g., Teratology Society, Toxicology Forum, American College of Veterinary Pathologists, Society of Environmental Toxicology and Chemistry, American College of Toxicology). Dr. DeSesso is on the editorial board of *Reproductive Toxicology*. He has published extensively in his areas of expertise, with his publications numbering well over 100.

Dr. DeSesso is a core panel member. He was selected for the core panel because of his experience in reproductive and developmental toxicity, in teratology, and in risk assessment.

### Disclosure

During the past six years, as an employee of Mitretek Systems, Dr. DeSesso has worked on several projects for companies that are sponsors of the ethylbenzene submission. None of these projects involved ethylbenzene.

*TERA* has determined that Dr. DeSesso has no conflicts of interest. Mitretek Systems' past activities with ethylbenzene sponsors are being disclosed to assure transparency. *TERA* does not believe these activities will impair Dr. DeSesso's scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. Penny Fenner-Crisp

Dr. Fenner-Crisp retired from her position as the Executive Director of the ILSI Risk Science Institute (RSI) in 2004 and established a private consulting practice. She had joined ILSI in 2000 from U.S. Environmental Protection Agency (EPA), having served as Senior Science Advisor to the Director, the Deputy Director of the Office of Pesticide Programs, and the Director of its Health Effects Division. She also was Special Assistant to the Assistant Administrator for Prevention, Pesticides and Toxic Substances, Director of the Health and Environmental Review Division of the Office of Pollution Prevention and Toxics, and Senior Toxicologist in the Health Effects Branch of the Office of Drinking Water. She served on several Risk Assessment Forum Technical Committees developing Agency-wide risk assessment guidelines. She was a co-chair of the Reference Dose Workgroup and a charter member and Chair of the Risk Assessment Forum. She played a key role in the development of several policies, including the policy guidance for use of Monte Carlo analyses in exposure assessment, the cumulative risk conceptual framework, and implementation of the cancer guidelines. Dr. Fenner-Crisp is an expert on World Health Organization International Programme on Chemical Safety (WHO IPCS) working groups charged with drafting Environmental Health Criteria documents, and planning the update/revision of the assessment principles used by the Joint Expert Committee on Food Additives and the WHO Expert Panel of the Joint Meeting on Pesticide Residues, on the WHO Expert Panel for the Joint Meeting on Pesticide Residues (nine years) and in activities related to the WHO IPCS project on Harmonization of Risk Assessment practices. She was U.S. Delegate to the Organization for Economic Co-operation and Development (OECD) Endocrine Disruptor Testing and Assessment workgroup and Mammalian Validation subgroup, and to the Expert Consultation on Acute Toxicity. She received the Fitzhugh Green Award, the Agency's highest award for contributions on behalf of EPA for its international activities.

Dr. Fenner-Crisp has a B.S. in Zoology from the University of Wisconsin-Milwaukee and an M.A. and Ph.D. in Pharmacology from University of Texas Medical Branch Galveston. Her research interests are in neuro- and cardiovascular pharmacology. She did a postdoctoral fellowship at Georgetown University Schools of Medicine and Dentistry.

Dr. Fenner-Crisp belongs to the Society of Toxicology (SOT) and is President of SOT's Risk Assessment Specialty Section. She is a member of the Society for Risk Analysis (SRA) and the National Capitol Area Chapter of SRA. She received SRA's first Risk Practitioner award. She is a Diplomate of the American Board of Toxicology, serving on its Board of Directors, and she was a member of the Board of Directors of the Toxicology Forum. She served on EPA's Endocrine Disruptor Screening and Testing Advisory Committee, EPA's Endocrine Disruptor Methods Validation Subcommittee, and the Strategic Science Team of the American Chemistry Council (ACC). She served as a member of the Board of Directors of the not-for-profit Midwest Center for Environmental Science and Public Policy until December 2006, and she was a member of the National Academies of Sciences. Currently, she is a member of the Drinking Water Committee of EPA's Science Advisory Board and EPA's National Pollution Prevention and Toxics Advisory Committee and of the Board of Directors of GreenBlue, a not-for-profit organization in Charlottesville, VA.

Dr. Fenner-Crisp is an *ad hoc* panel member. She was selected for the ethylbenzene panel because of her experience in evaluating the relevance of modes of action of animal toxicity to human risk assessment.

**Disclosure**

Dr. Fenner-Crisp was a public member of the ACC's Long-range Research Initiative Strategic Science Team from 2002 to 2005; this work did not involve ethylbenzene specifically, or VCCEP.

*TERA* has determined that Dr. Fenner-Crisp has no conflicts of interest. Her previous relationships with ACC are being disclosed to assure transparency. *TERA* does not believe Dr. Fenner-Crisp's previous activities will impair her scientific objectivity as a VCCEP ethylbenzene panel member.

## Dr. Pertti (Bert) Hakkinen

Dr. Hakkinen is a Principal of the Gradient Corporation, and leads its Product Safety practice. Formerly, he was on the staff of the European Commission (EC) at the EC Joint Research Centre in the Physical and Chemical Exposure Unit of the Institute for Health and Consumer Protection. While at the EC, he helped develop and manage work packages for EIS-ChemRisks, the European Information System on risks from chemicals released from consumer products and articles (textiles, toys, etc.).

Dr. Hakkinen is a member of the Scientific Advisory Panel of the (U.S.) Mickey Leland National Urban Air Toxics Research Center and has served as the vice chair of this panel since March 2003. Prior to joining the EC staff, Dr. Hakkinen was on the staff of Toxicology Excellence for Risk Assessment (*TERA*). Before joining *TERA*, he worked at the Procter & Gamble Company to provide global human exposure and risk assessment support for numerous types of consumer products and chemicals. While at Procter & Gamble, he chaired the Exposure Assessment Task Group of the Chemical Manufacturers Association (now the American Chemistry Council [ACC]) for several years, and was a chair of the ACC's Human Exposure Assessment Technical Implementation Panel.

Dr. Hakkinen earned a B.A. in Biochemistry and Molecular Biology from the University of California, Santa Barbara, and received his Ph.D. in Comparative Pharmacology and Toxicology from the University of California, San Francisco. He served as a postdoctoral investigator in respiratory toxicology, and exposure and risk assessment at the Biology Division of the Oak Ridge National Laboratory. Dr. Hakkinen has been an invited expert or reviewer for the U.S. EPA, Health Canada, and other associations to develop or revise human exposure assessment guidance, resource documents, and software. He has lectured on exposure and risk assessment, risk perception, and risk communication at the University of Cincinnati and elsewhere.

Dr. Hakkinen is a member of the Society of Toxicology (SOT) and a charter member of the Society for Risk Analysis (SRA) and the International Society of Exposure Analysis (ISEA). He proposed the idea for the *Residential Exposure Assessment: A Sourcebook*, developed and published in 2001 via the expertise and involvement of members of SRA's Exposure Assessment Specialty Group, ISEA members, and many others. Dr. Hakkinen received SRA's Outstanding Service Award in 1996. He was on the editorial board of *Toxicology* and was a co-editor and co-author of the latest edition of *Information Resources in Toxicology* and is a co-editor and co-author of the new edition under development. Further, he is a co-editor and co-author of the latest edition (2005) of the *Encyclopedia of Toxicology*. Dr. Hakkinen has authored and co-authored numerous other publications, including ones on consumer product exposure and risk assessments, consumer risk perceptions, toxicological interactions, respiratory tract toxicology, and computer software and databases.

Dr. Hakkinen is a core panel member. He was selected for the core panel because of his experience in evaluating chemical exposures, especially to consumer product ingredients, and also because of his experience in toxicology and risk assessment.

### Disclosure

Dr. Hakkinen chaired ACC task groups and panels in 2001 and earlier, while employed by the Procter and Gamble Company. His current employer, Gradient, has had projects with several of the ethylbenzene sponsors, but the projects were unrelated to ethylbenzene. Gradient was a recent (January 2007) co-sponsor of an ACC and Canadian Chemical Producers' Association (CCPA) "REACH" workshop held to provide intensive implementation training, and Dr. Hakkinen was a speaker at this workshop. It did not involve ethylbenzene.

*TERA* has determined that Dr. Hakkinen has no conflicts of interest. His chairing of ACC task groups and panels, Gradient's work for ACC and ethylbenzene sponsors in the past, and Gradient's recent sponsorship of a ACC and CCPA workshop are being disclosed to assure transparency. *TERA* does not believe these activities will impair Dr. Hakkinen's scientific objectivity as a VCCEP ethylbenzene panel member.

**Dr. Michael Jayjock**

Dr. Jayjock is a Senior Analyst with The LifeLine Group, a non-profit organization dedicated to the development of scientific tools for human exposure and risk assessment. He has been with LifeLine for 3 years. Previous to this he was a Senior Research and Environmental Health and Safety Fellow and Manager for Risk Assessment at the Rohm and Haas Company; and had been working with that company for 35 years. In his current position, he is responsible for the determination of human health risk from and development of tools for the evaluation of human exposure and risk to chemicals.

Dr. Jayjock received both his Ph.D. in Environmental Engineering and his M.S. in Environmental Science and Occupational Health from Drexel University. He is a Fellow of the American Industrial Hygiene Association and is certified in the Comprehensive Practice of Industrial Hygiene by the American Board of Industrial Hygiene.

Dr. Jayjock's professional activities include such areas as exposure modeling research, human exposure and risk assessment to environmental pollutants, and uncertainty analysis. He has published extensively in peer-reviewed publications and served from 1998-2003 as an Editorial Board Member for the *American Industrial Hygiene Journal.* He has made numerous technical presentations, including at the American Industrial Hygiene Conference, International Society of Exposure Assessment Conference, and the Air Toxics Monitoring Workshop to Support the U.S. Environmental Protection Agency's (EPA) Integrated Urban Air Toxics Strategy. His wide service on advisory committees includes: EPA Board of Scientific Councilors Peer Review Panel for Office of Research and Development Science Program, Executive Committee, Human Health Research Strategy Panel; EPA Office of Pollution Prevention & Toxics Voluntary Children's Chemical Evaluation Program (VCCEP), Peer Consultation Panels on Flame Retardants and on Methyl Ethyl Ketone; EPA Science Advisory Board, Executive Committee, Human Health Research Strategy Panel; EPA Science Advisory Board Consultant - Integrated Human Exposure Committee; EPA Science Advisory Board Member - Integrated Human Exposure Committee (IHEC); and National Research Council - National Academy of Sciences, as a Member of the Committee to Review Risk Management in the U.S. Department of Energy's (DOE) Environmental Remediation Program, the Committee on Advances in Assessing Human Exposure to Airborne Pollutants, and the Committee on Toxicology – Subcommittee on Risk Assessment of Flame-Retardant Chemicals.

Dr. Jayjock also serves as a team teacher or guest lecturer for local universities including Drexel, the Philadelphia University of the Sciences, Temple University, and Thomas Jefferson University. He is a guest lecturer at the University of Pennsylvania Medical School, Residency Program for Occupational Medicine; and he is also an instructor for a professional development course on risk assessment for the American Industrial Hygiene Conference and Exposition. Previously, he served as course director and instructor for Risk Assessment and Intermediate Exposure Modeling at the University of North Carolina Education Research Center, Summer Institute.

Dr. Jayjock is an *ad hoc* panel member. He was selected for the ethylbenzene panel because of his expertise and experience in using multiple tools to determine chemical exposures and applying the findings to human risk assessment.

**Disclosure**

Dr. Jayjock chaired ACC task groups and panels in 2002 and earlier, while employed by the Rohm and Haas Company. He is currently a subcontractor for LINEA, Inc. Other persons in LINEA, Inc have contributed to previous VCCEP projects, but they have not provided work on ethylbenzene. *TERA* has determined that Dr. Jayjock has no conflicts of interest.

## Dr. Virginia (Ginger) Moser

Dr. Moser is a Research Toxicologist in the Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development of the U.S. Environmental Protection Agency (EPA) located in Research Triangle Park, North Carolina. She also served as Acting Branch Chief for the Neurobehavioral Toxicology Branch of the Neurotoxicology Division for four years. Current research pursuits include evaluating neurotoxicity of pesticide mixtures, unique susceptibilities of the young to neurotoxicants, and mechanisms of neurotoxic environmental chemicals.

Dr. Moser received her B.S. in Pharmacy from the University of North Carolina at Chapel Hill, and her Ph.D. in Pharmacology and Toxicology at the Medical College of Virginia. She has led an active research program at the EPA since joining the division as a National Research Council postdoctoral fellow in 1983. She holds adjunct faculty positions at the Integrated Toxicology Program at Duke University and the Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University.

Dr. Moser is a Diplomate of the American Board of Toxicology, and served on the Board of Executive Directors and as Treasurer of that organization. She has received several honors from EPA, including the Scientific Achievement Award for Human Health Research for her pioneering efforts in developing the US EPA functional observational battery, as well as bronze medals and technical achievement awards. She is a member of the EPA Risk Assessment Forum, and is currently working on panels addressing Data-Derived Uncertainty Factors and Mode of Action Harmonization. As an active member of numerous scientific societies, she has held office, served on planning committees, and organized meetings. Currently, she is President-Elect of the North Carolina Regional Chapter of the Society of Toxicology. In addition, she serves as Neurotoxicology Section Editor for *Drug and Chemical Toxicology*, and is on the editorial board of three additional journals. She has over 100 peer-reviewed manuscripts and book chapters published or in press.

Dr. Moser has been instrumental in validating and promoting the use of neurobehavioral test methods for toxicity screening, specifically the functional observational battery, motor activity, and developmental neurotoxicity. She helped shape the test guidelines for neurobehavioral toxicity testing promulgated by the EPA, the Organization for Economic Co-operation and Development (OECD), and the U.S. Food and Drug Administration (FDA), and served as Study Director for a large, international multi-laboratory Collaborative Study on Neurobehavioral Test Methods. She has also been involved with training personnel in contract, chemical, and pharmaceutical testing laboratories in the conduct of these methods, as well as training risk assessors in the interpretation of those data.

Dr. Moser is an *ad hoc* panel member. She was selected for the ethylbenzene panel because of her broad experience in neurotoxicology and risk assessment and her expertise in evaluating treatment-related effects in developmental neurotoxicity testing.

### Disclosure

Dr. Moser is employed by the EPA, which has taken public positions on the VCCEP pilot chemicals, including ethylbenzene. The comments that Dr. Moser makes during this meeting are her personal opinions, and her opinions should not be construed to represent the opinions of the EPA.

*TERA* has determined that Dr. Moser has no conflicts of interest.

## Dr. Garold Yost

Dr. Yost is a Professor of Pharmacology and Toxicology at the University of Utah. He received his Ph.D. in Organic Chemistry from Colorado State University and subsequently worked as a postdoctoral fellow and a research scientist. His training ranged from natural products to cytochrome P450 mechanisms and inactivation and mass spectrometry of drug metabolites. Dr. Yost spent six years at the College of Pharmacy, Washington State University, and then moved to the University of Utah, College of Pharmacy, Department of Pharmacology and Toxicology, where he was promoted to Professor of Pharmacology and Toxicology and Adjunct Professor of Medicinal Chemistry. His primary research interests are focused on the elucidation of the chemical, biochemical, and cellular mechanisms of toxicity to lung tissues caused by exposure to environmental pollutants. He also studies the mechanisms of cytochrome P450-mediated bioactivation of toxicants and the mechanisms by which certain genes are selectively expressed in lung tissues. Dr. Yost is an internationally recognized authority on toxicology in the respiratory tract, with expertise on cytochrome P450-mediated mechanisms of lung injury. He received the Zeneca Traveling Lectureship and the Colgate-Palmolive Traveling Lectureship from the Society of Toxicology.

Dr. Yost served as Secretary of the International Society for the Study of Xenobiotics, on the National Academy of Sciences' Committee on Toxicology, and is currently the Chairman of the Subcommittee on Spacecraft Water Exposure Guidelines for the Academy. Dr. Yost has served on several study sections for the National Institutes of Health (NIH) and was a member of the Environmental Health Sciences Review committee for National Institute for Environmental Health Sciences (NIEHS). He is an elected Fellow and Member of the Board of Directors of the Academy of Toxicological Sciences. He currently serves as an elected member of the Executive Committee, Division of Chemical Toxicology of the American Chemical Society. He is an Associate Editor of the journal *Drug Metabolism and Disposition.* He is on the editorial boards of *Toxicology and Applied Pharmacology*, *Chemical Research in Toxicology*, *Journal of Toxicology and Environmental Health*, *Chemico-Biological Interactions*; *Drug Metabolism and Disposition*, and *Toxicological Sciences*.

Dr. Yost heads a drug metabolism and lung diseases group of research scientists. He currently has five NIH grants and a U.S. Environmental Protection Agency (EPA) grant in his research group. He has published 90 papers in the peer-reviewed primary literature, along with 121 abstracts and 12 book chapters. The work in his laboratory is focused on environmental pollutants that contribute to adverse effects in lung tissues. Dr. Yost is investigating the mechanisms responsible for human lung disease caused by particulate matter in air pollution. He has cloned, expressed, and characterized multiple "irritant receptors" expressed on human lung epithelial cells and activated by particulates in polluted air, and by capsaicinoids.

Dr. Yost is an *ad hoc* panel member. He was selected for the ethylbenzene panel because of his experience in pulmonary toxicology and metabolism and the mode of action of tumor formation.

### Disclosure

None

*TERA* has determined that Dr. Yost has no conflicts of interest.

## VCCEP Ethylbenzene Presenter Biosketches

**Dr. Marcy Banton**
Manager, Toxicology
Lyondell Chemical Company

Dr. Banton is Manager of Toxicology at Lyondell Chemical Company. Lyondell Chemical Company**,** headquartered in Houston, Texas, is a major manufacturer of basic chemicals and derivatives including ethylene, propylene, titanium dioxide, styrene, ethylbenenze, polyethylene, propylene oxide and acetyls and is a refiner of heavy, high-sulfur crude oil and a significant producer of gasoline-blending components. Dr. Banton has more than 20 years of experience in toxicology and health and environmental risk assessment, has participated and chaired toxicology Panels within the American Chemistry Council, the American Petroleum Institute, the Synthetic Organic Chemicals Manufacturing Association, the European Chemical Industry Council (CEFIC), the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), and the Health and Environmental Sciences Institute (ILSI-HESI), and authored or co-authored more than 50 papers or abstracts in the fields of toxicology and risk assessment. She is a board certified veterinary toxicologist (Diplomate of the American Board of Veterinary Toxicology) and a member of the Society of Toxicology, the Society of Risk Analysis, and the American Veterinary Medical Association. Dr. Banton received a D.V.M. and Ph.D. in Toxicology from Louisiana State University.

**Dr. James Bus**
Director
The Dow Chemical Company

Dr. Bus is Director of External Technology, Toxicology and Environmental Research and Consulting at The Dow Chemical Company. Dow is a diversified chemical company that harnesses the power of innovation, science and technology to constantly improve what is essential to human progress. Dr. Bus participates in several external institutions including: Member of the Board of Directors and Science Program Committee of CIIT Centers for Health Research; the American Chemistry Council and ICCA Long-Range Research Initiatives; the EPA Science Advisory Board; the FDA NCTR Science Advisory Board; and several National Academy of Sciences/National Research Council Committees including the Standing Committee on Emerging Issues and Data on Environmental Contaminants and the Board on Environmental Sciences and Toxicology (BEST). Dr. Bus is a member of the Society of Toxicology (serving as President in 1996-97), the American Society for Pharmacology and Experimental Therapeutics, the American Conference of Governmental and Industrial Hygienists, the Teratology Society. He is a Diplomate and Past-President of the American Board of Toxicology and a Fellow, The Academy of Toxicological Sciences. Dr. Bus received the Society of Toxicology Achievement Award (1987), Rutgers University Robert A. Scala Award (1999), and K.E. Moore Outstanding Alumus Award (Michigan State University, Dept. Pharmacol. and Toxicol.). He received his Ph.D. in pharmacology from Michigan State University and currently is an Adjunct Professor in the Dept. Pharmacology and Toxicology in that institution. His research interests include the mechanism of oxidant toxicity, defense mechanisms to toxicant exposure, and relationships of pharmacokinetics to expression of chemical toxicity.

**Dr. Michael Gargas**
Managing Principal
The Sapphire Group

Dr. Gargas is a Managing Principal with *The Sapphire Group™*, a risk assessment and risk management consulting firm. Dr. Gargas is a toxicologist with over 28 years of related environmental and health experience. He oversees and prepares human health risk assessments, conducts toxic tort support investigations, serves as an expert witness, interacts with regulatory agencies, and addresses critical toxicological issues through applied and basic research on behalf of clients. Dr. Gargas' area of expertise is in human health risk assessment and biochemical toxicology research with emphasis in the areas of inhalation toxicology, chemical metabolism, physiologically based pharmacokinetic (PBPK) modeling, and chemical dosimetry, with specific application of these approaches to risk assessments. Dr. Gargas completed his doctorate in Biomedical Sciences (Toxicology Specialty) from Wright State University. He has been an active member in the Society of Toxicology since 1989 and the Society for Risk Analysis since 1992 and has served on the editorial board of *Toxicology and Applied Pharmacology*. He is a member and has served as a Councilor to the Risk Assessment Specialty Section of the SOT and is currently serving as the President of that Specialty Section. He has published seven book chapters and over 70 peer-reviewed articles on a wide range of health and toxicologic topics. Dr Gargas is also an Adjunct Assistant Professor of Toxicology in the School of Medicine at Wright State University, serving as director for a yearly graduate course in biokinetics and toxicology.

**Dr. Janet Kester**
Toxicologist/Partner
NewFields LLC

Dr. Kester is an American Board of Toxicology-certified toxicologist and partner of NewFields LLC, an environmental consulting company headquartered in Atlanta, GA. Dr. Kester has seventeen years' experience in environmental risk analysis, including toxicology, ecological and human health exposure and risk assessment, risk communication, and litigation support. She has designed and performed exposure and risk assessments under a variety of regulatory jurisdictions in the U.S., Canada, Brazil, Venezuela, Guam, Indonesia, Japan, and Australia. She has published research and symposium papers and book chapters, and presented seminars and short courses on issues in toxicology and risk assessment in both the U.S. and abroad. She is a co-author of the ASTM's "Standard Guide for Risk-Based Corrective Action Applied at Petroleum Release Sites" (RBCA) and the "Standard Guide for Risk-Based Corrective Action" ('RBCA II'), and served as an ASTM-certified RBCA trainer. Dr. Kester developed and taught graduate courses in toxicology as an Adjunct Professor at the Rochester Institute of Technology and an Associate of Toxicology at the University of Rochester. She is a member of the American Chemical Society, International Society of Exposure Analysis, Society for Risk Analysis, Society of Environmental Toxicology and Chemistry, and Society of Toxicology. Dr. Kester received a B.S. from Cornell University, and M.S. and Ph.D. degrees from the University of Rochester.

**Dr. Elizabeth Moran**
Senior Director
American Chemistry Council

Dr. Moran is Senior Director, Chemical Products and Technology Division of the American Chemistry Council. Dr. Moran is currently the Manager of the Ethylbenzene Panel and the Olefins Panel. Over the past 25 years, Dr. Moran has managed a number of Panels at the American Chemistry Council covering a range of chemicals including phthalate esters, carbon disulfide, ketones, glycol ethers, propylene oxide, and methylenedianiline. Prior to joining ACC, she was a program manager at JRB Associates, where she provided toxicology consulting to the USEPA. Earlier, Dr. Moran directed the Flavor Safety Program at International Flavors and Fragrances, Inc., where she was responsible for toxicology testing on new chemicals. Previously, Dr. Moran conducted lipid nutrition research at the US Department of Agriculture and was a visiting professor at the University of Maryland. Dr. Moran received her Ph.D. in biochemistry from the University of Maryland and is certified in toxicology by the American Board of Toxicology. Dr. Moran is a member of the American College of Toxicology and the American Chemical Society.

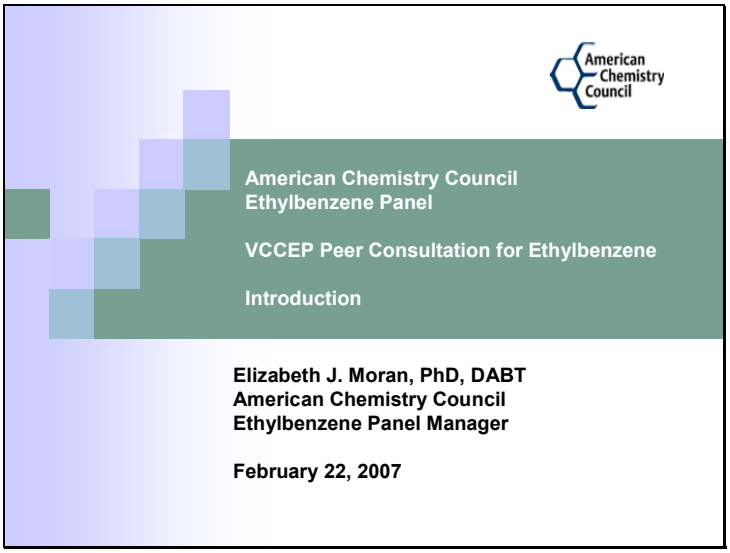
**Dr. Lisa Sweeney**
Program Manager
The Sapphire Group

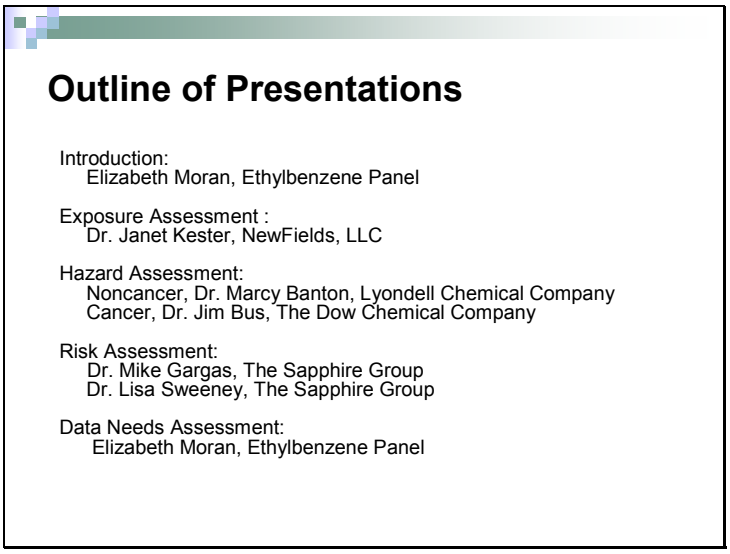
Dr. Sweeney is a toxicologist with a broad range of experience in the application of toxicology, chemistry, and engineering to problems in the health and environmental sciences. She has over 10 years experience in risk assessment, pharmacokinetics, and biochemical engineering and her responsibilities involve applied toxicology, including the development and refinement of physiologically-based pharmacokinetic (PBPK) models and their application to risk assessment. Compounds with which she has experience include 1,3-butadiene, isoprene, ethylene oxide, propylene oxide, ethylene glycol ethers, ethyl acrylate, ethylene glycol, ethylene dichloride, ethylbenzene, naphthalene, acrylonitrile, chromium, propanol, 1,4-dioxane, dichloromethane, iodomethane, 1,1,2-trichloroethane, perchloroethylene, and total petroleum hydrocarbons (TPH). She is a board certified toxicologist (Diplomate of the American Board of Toxicology) and Certified Hazardous Materials Manager (CHMM) and a member of the Society of Toxicology, American Chemical Society, Tau Beta Pi, and the Ohio Society for Risk Analysis. Dr. Sweeney received her bachelor's degree in Chemical Engineering from Case Western Reserve University and her doctorate in Chemical Engineering with a minor in Toxicology from Cornell University.

# Appendix C

# Voluntary Children's Chemical Evaluation Program (VCCEP)
# Peer Consultations on Ethylbenzene
# February 22-23, 2007

## Sponsors' Presentation Slides


American Chemistry Council
Ethylbenzene Panel
VCCEP Peer Consultation for Ethylbenzene
Introduction
Elizabeth J. Moran, PhD, DABT
American Chemistry Council
Ethylbenzene Panel Manager
February 22, 2007


Outline of Presentations
Introduction:
Elizabeth Moran, Ethylbenzene Panel
Exposure Assessment :
Dr. Janet Kester, NewFields, LLC
Hazard Assessment:
Noncancer, Dr. Marcy Banton, Lyondell Chemical Company
Cancer, Dr. Jim Bus, The Dow Chemical Company
Risk Assessment:
Dr. Mike Gargas, The Sapphire Group
Dr. Lisa Sweeney, The Sapphire Group
Data Needs Assessment:
Elizabeth Moran, Ethylbenzene Panel

## Ethylbenzene VCCEP Sponsor Companies

Chevron Phillips Chemical Company LP
The Dow Chemical Company
GE Plastics
INEOS Styrenics
Lyondell Chemical Company
NOVA Chemicals Inc.
Sterling Chemicals, Inc.
TOTAL Petrochemicals USA, Inc.

## VCCEP Selection Basis

- Ethylbenzene was selected for the VCCEP pilot program based on:
  - Detection in Human Blood (NHANES)
  - Detection in expired air (TEAM)
  - Detection in Ground Water (EPA, others)
  - Availability of Hazard Data (OECD SIDS, others)

## Sources of Ethylbenzene

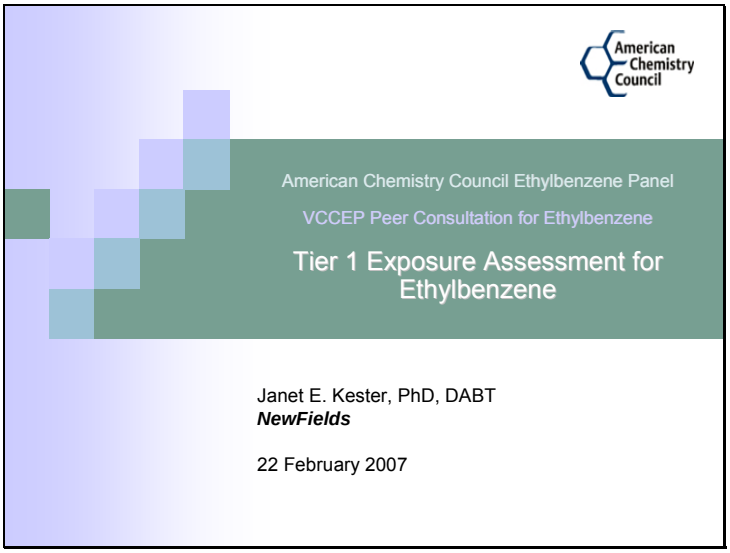
- Refinery Chain of Commerce – by product of petroleum refining, component of gasoline and other petroleum products, and mixed xylene solvent
- Ethylbenzene/Styrene Chain of Commerce – industrial chemical used in the production of styrene and styrene products
  - Primarily produced from benzene and ethylene
  - 8 current manufacturers in the US
- Component of Combustion

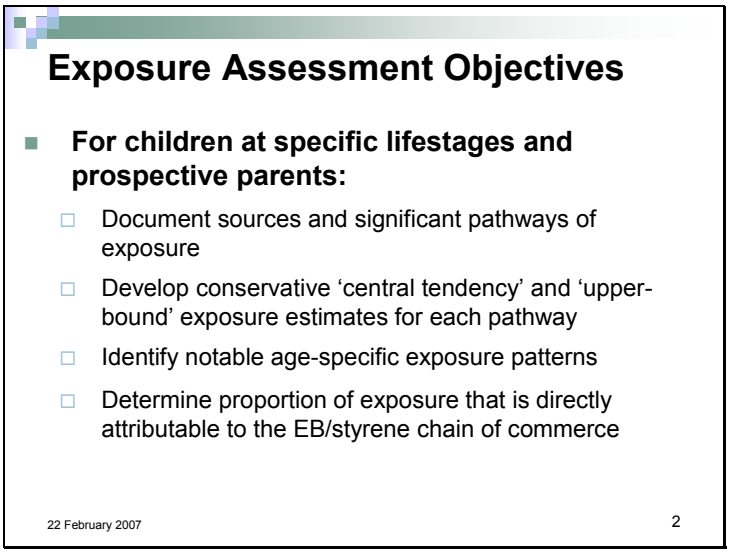
## Previous Assessments of Ethylbenzene

- IRIS Assessment in 1991, currently being updated
- IPCS assessment in 1996
- ATSDR assessment in 1999
- IARC assessment in 2000
- OECD SIDS assessment in 2002
- EU Risk Assessment in development
- AEGL in development

## Regulatory Overview

- Ethylbenzene is regulated under a number of environmental health regulations, including:
  - Clean Air Act – Stationary Sources, mobile sources and as a VOC
  - Clean Water Act, Safe Drinking Water Act
  - Toxics Release Inventory (TRI) reporting


American Chemistry Council
American Chemistry Council Ethylbenzene Panel
VCCEP Peer Consultation for Ethylbenzene
Tier 1 Exposure Assessment for Ethylbenzene
Janet E. Kester, PhD, DABT
NewFields
22 February 2007


Exposure Assessment Objectives
For children at specific lifestages and prospective parents:
Document sources and significant pathways of exposure
Develop conservative 'central tendency' and 'upper-bound' exposure estimates for each pathway
Identify notable age-specific exposure patterns
Determine proportion of exposure that is directly attributable to the EB/styrene chain of commerce
22 February 2007
2

## Sources of Ethylbenzene

- **Refinery Chain of Commerce**
  - Byproduct of petroleum refining
  - Component of gasoline and other petroleum products
  - Mixed xylene solvent
- **EB/Styrene Chain of Commerce**
  - Industrial chemical used in the production of styrene and styrene products
  - Primarily produced from benzene and ethylene
  - 8 current manufacturers in the US
- **Component of Combustion**

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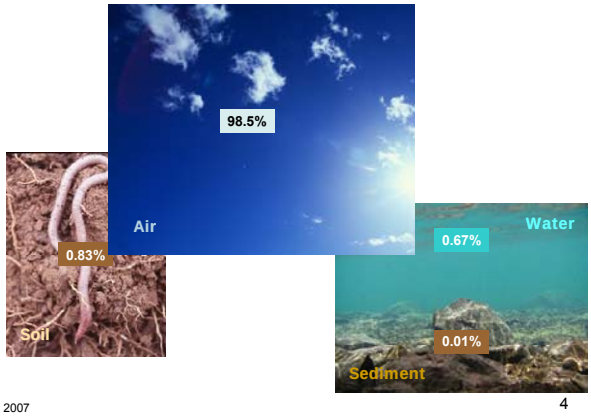
## Environmental Transport and Partitioning

**Mackay Level III Fugacity modeling**

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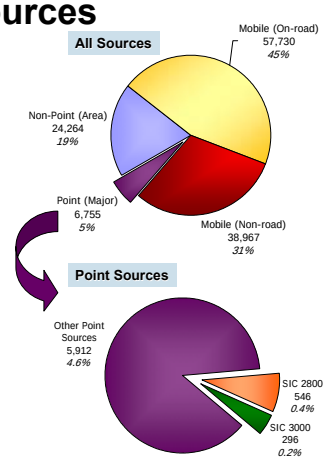
## Exposure Data Sources

**Environmental releases**

- **National Emissions Inventory (NEI)**
  - Point (major sources)
  - Area (non-point)
  - Mobile
  - EB/styrene producers (in SIC codes 28 [“Chemicals”] and 30 [“Rubber and Plastics”]) generate less than 1% of total emissions
- **Toxics Release Inventory (major industrial sources)**




Data from NEI 2002



## Exposure Data Sources

- **Ambient air**
  - EPA AirData
    - Urban
    - Rural/suburban
  - Published studies
- **Potable surface and groundwater**
  - ATSDR HazDat
  - USGS National Water Quality Assessment Program (NAWQA)
  - National Contaminant Occurrence Database (NCOD)
  - Published studies
- **Indoor air**
  - Published studies
    - Homes
    - Schools
    - Commercial buildings
    - Motor vehicles
    - I/O ratios
  - “Occupational Exposures to Ethylbenzene During Styrene Manufacturing” (provided by ACC)
- **Soil and Sediment**
  - ATSDR HazDat
  - EPA Storage and Retrieval Database (STORET)

## Exposure Data Sources

- **Food**
  - Volatile Compounds in Food (VCF) database
  - UK Total Diet Study
  - FDA Market Basket Study
  - Published studies
  - Modeled migration from styrenic food-contact materials
- **Human milk**
  - PBPK modeled
- **Household products**
  - Sack database
  - EPA Source Ranking Database
  - National Library of Medicine Household Products Database
  - Published studies
- **Polystyrene toys**
  - Modeled migration due to mouthing

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## Selection of Exposure Media

| Potential Exposure Medium | Selected for Quantitative Analysis? | Rationale |
|---|---|---|
| **Air** | Yes | ■ Predominant environmental compartment<br>■ Abundant national data<br>■ Frequently detected |
| **Water** | No | ■ Minor environmental compartment<br>■ Abundant national data<br>■ Rarely detected, low concentrations |
| **Soil/sediment** | No | ■ Minor environmental compartment<br>■ Rarely detected, low concentrations<br>■ Non-persistent |
| **Food** | Yes | ■ Non-bioaccumulative<br>■ Seldom detected in fresh food<br>■ Present in some foods due to migration from air sources or styrenic food packaging |
| **Human milk** | Yes | ■ Child-specific exposure medium |
| **Polystyrene toys** | Yes | ■ Child-specific exposure medium |

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## Receptor Characterization

Grouped by physiological and behavioral lifestages

(similar but not identical to recent EPA *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants*)

- **Children**
  - <1
    - Bottle-fed
    - Breastfed
  - 1-2
  - 3-5
  - 6-8
  - 9-14
  - 15-19
- **Prospective Parents (20-45)**
  - At-home parent
  - Production worker
  - Office worker

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## Microenvironments

- **Outdoors**
- **Indoors**
  - Home
  - School
  - Motor vehicle
  - Work (office)
  - Work (production)

Urban — Smoking

Rural/ Suburban — Non-smoking

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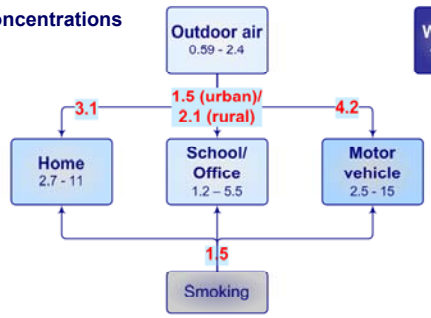
## Exposure Concentrations: Air

- **I/O ratios applied to urban/rural ambient data to estimate levels in indoor environments**
- **Additional factor for ETS**

**Ranges of EB Concentrations in Air (μg/m³)**

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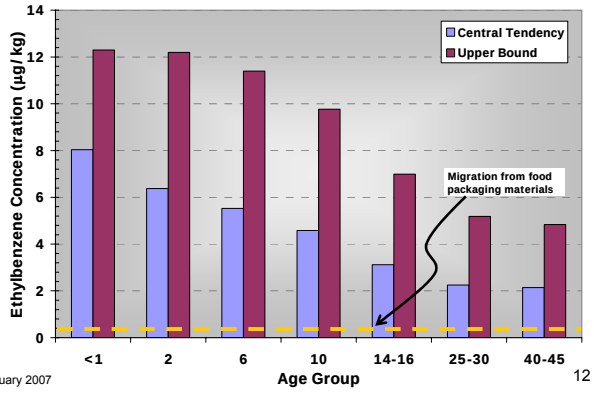
## Exposure Concentrations: Food

Mean and maximum concentrations and age- and food-specific intake rates from FDA Total Diet Study used for central tendency and upper-bound estimates

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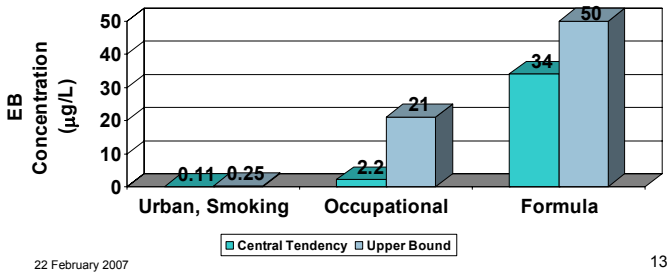
## Exposure Concentrations: Human Milk

- Unique exposure pathway for infants 0 to <1
- No published data available; used PBPK model to estimate central tendency and upper-bound concentrations in milk of general population (urban smokers) and production workers based on diet and daily activities

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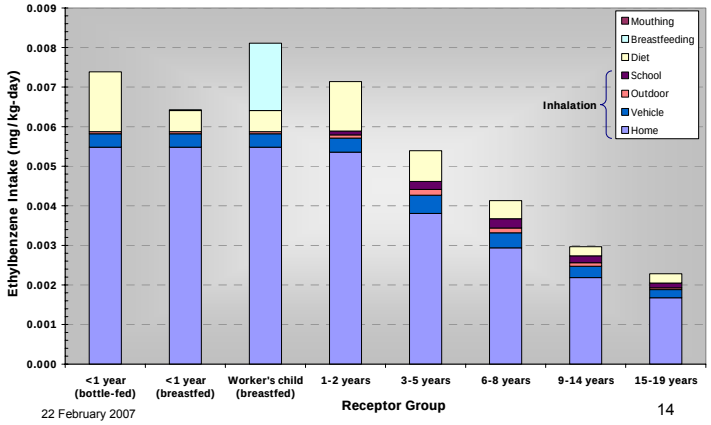
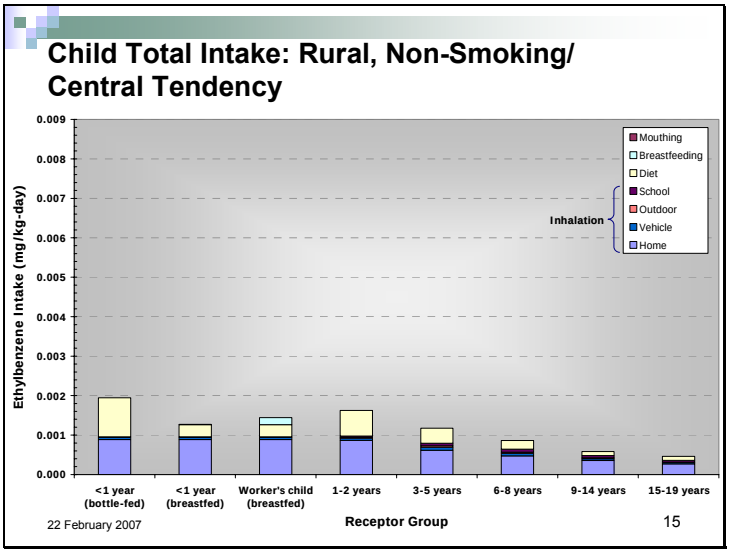
## Child Total Intake: Urban, Smoking/ Upper-Bound




22 February 2007

14


Child Total Intake: Rural, Non-Smoking/ Central Tendency
Ethylbenzene Intake (mg/kg-day)
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Mouthing
Breastfeeding
Diet
School
Outdoor
Vehicle
Home
Inhalation
<1 year (bottle-fed)
<1 year (breastfed)
Worker's child (breastfed)
1-2 years
3-5 years
6-8 years
9-14 years
15-19 years
22 February 2007
Receptor Group
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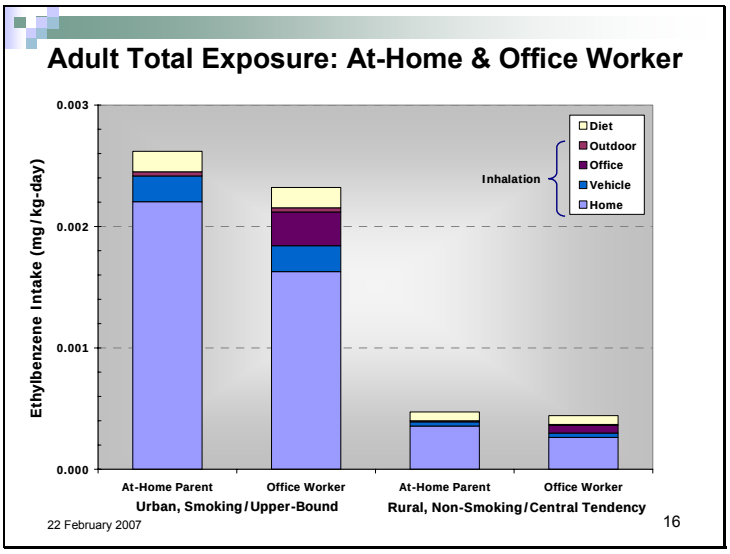
Adult Total Exposure: At-Home & Office Worker
Ethylbenzene Intake (mg/kg-day)
0.003
0.002
0.001
0.000
Diet
Outdoor
Office
Vehicle
Home
Inhalation
At-Home Parent
Office Worker
At-Home Parent
Office Worker
Urban, Smoking/Upper-Bound
Rural, Non-Smoking/Central Tendency
22 February 2007
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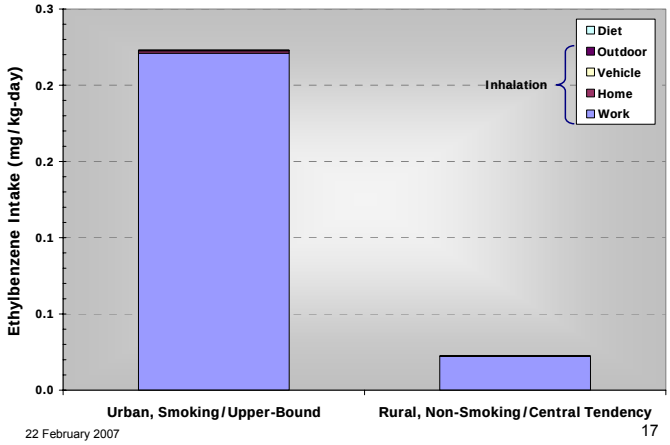
## Adult Total Exposure: Production Worker






## Conclusions

- **Inhalation is dominant exposure pathway for all lifestages**
- **Robust air database is adequate for exposure characterization**
  - Concentrations in ambient air are generally low (<1 ppb) and decreasing
  - Contribution of the EB/styrene chain of commerce to total inhalation exposures is around 1%
- **Concentrations in food are low, and migration from packaging contributes <25%**
- **Production workers have by far the highest exposure, but for the rest of us, air in the home is the dominant exposure medium**
- **Young children have higher exposures than older children and non-occupationally exposed adults**
  - Bottle-fed greater than breastfed (except for worker's child)
  - Top contributing foods for the bottle-fed infant are formula and whole milk

American Chemistry Council

**American Chemistry Council Ethylbenzene Panel**

**VCCEP Peer Consultation for Ethylbenzene**

**Presentation on Hazard Assessment**

**Marcy I. Banton, DVM, PhD, DABVT**
**Lyondell Chemical Company**
**Chair ACC EB Panel VCCEP Research Task Group**

**February 22, 2007**

**Voluntary Children's Chemical Evaluation Program Pilot – Ethylbenzene Studies**
**(New Panel-sponsored studies in bold)**

| Tier 1 | Tier 2 | Tier 3 |
|---|---|---|
| Acute toxicity | Subchronic toxicity | **Neurotoxicity screening battery** |
| Repeated dose toxicity with reproductive and developmental toxicity screens | Prenatal developmental toxicity<br>**Reproductive and fertility effects** | Carcinogenicity |
| Bacterial reverse mutation assay | **Immunotoxicity** | **Developmental neurotoxicity** |
| *In vitro* or *in vivo* chromosomal aberrations or *in vivo* micronucleus test | *In vivo* chromosomal aberrations or *in vivo* micronucleus test | |
| | Metabolism and pharmacokinetics | |

## Voluntary Children's Chemical Evaluation Program Pilot – Ethylbenzene Hazard Assessment

| Tier 1 | Health Effect Endpoint | Effect/No Effect Level |
|---|---|---|
| Acute toxicity | Mortality | Oral LD50 = 5.46 g/kg bwt<br>Dermal LD50 = 15.3 g/kg bwt<br>Inhalation LC50 = 4000 ppm |
| Repeated dose toxicity with reproductive and developmental toxicity screens | | Superseded by Tier 2 90-Day Subchronic Toxicity studies, a 2-Gen Reproductive Toxicity study, and Developmental Toxicity studies |
| Bacterial reverse mutation assay | Mutations | Negative Results<br>(Variable Results in Mouse Lymphoma Cells. Non-Mutagenic in Recent Guideline Study) |
| *In vitro* chromosomal aberrations | Chromosome Damage | Negative for Sister Chromatid Exchanges and Chromosome Aberrations in Chinese Hamster Ovary Cells |

## Voluntary Children's Chemical Evaluation Program Pilot – Ethylbenzene Hazard Assessment

| Tier 2 | Health Effect Endpoint | Effect/No Effect Level |
|---|---|---|
| Subchronic toxicity | Liver and Kidney Weights<br>Blood, GI, Kidney and Liver | NOAEL = 1000 ppm<br>NOAEL = 75 mg/kg bwt |
| Prenatal developmental toxicity | Mild increases in fetal effects with mild maternal increases in organ weights | NOAEL = 500 ppm |
| Reproductive and fertility effects | Reproductive effects | NOAEL = 500 ppm |
| Immunotoxicity | Humoral immune system | NOAEL = 500 ppm |
| *In vivo* micronucleus test | Chromosome damage | Negative |
| Metabolism and pharmacokinetics | | Well absorbed from skin, lungs and GI tract, rapidly distributed in the body, metabolized primarily via hydroxylation of the 2 carbons of the side-chain and then further oxidized to a range of metabolites that are excreted principally in the urine |

**Voluntary Children's Chemical Evaluation Program Pilot – Ethylbenzene Hazard Assessment**

| Tier 3 | Health Effect Endpoint | Effect/No Effect Level |
|---|---|---|
| Neurotoxicity screening battery | Adult nervous system | NOAEL = 500 mg/kg bwt |
| | Ototoxicity<br>Audiometric Threshold<br>Outer Hair Cell Loss | <br>NOAEL = 200 ppm<br>LOAEL = 200 ppm |
| Carcinogenicity | Liver, kidney, lung and testes tumors | NOAEL = 250 ppm |
| Developmental neurotoxicity | Neurodevelopment | NOAEL = 500 ppm |

## Summary

- Ethylbenzene toxicity well characterized (all three tiers of tests addressed)
  - Data are adequate for hazard assessment
- Animal toxicity observed $\geq$ 200 ppm for noncancer and 750 ppm for cancer
- General population exposure is 1 – 2.5 ppb (average air concentrations)

American Chemistry Council

# American Chemistry Council Ethylbenzene Panel

## VCCEP Peer Consultation for Ethylbenzene

## Presentation on Cancer Mode of Action

**James S Bus, PhD, DABT, Fellow ATS**
**The Dow Chemical Company**
**ACC EB Panel, VCCEP Research Task Group**

**February 22, 2007**

## The Issue: NTP Carcinogenicity Bioassay (1999)

- F344 rats and B6C3F1 mice
- 0, 75, 250 and 750 ppm, 6 hr/day, 5 d/wk
- Responses:
  - Male/female rat: ↑ kidney tumors in males; lesser ↑ in females with step sectioning
  - Female mice: ↑ liver tumors
  - Male mice: ↑ lung tumors in terminal bronchiolar region

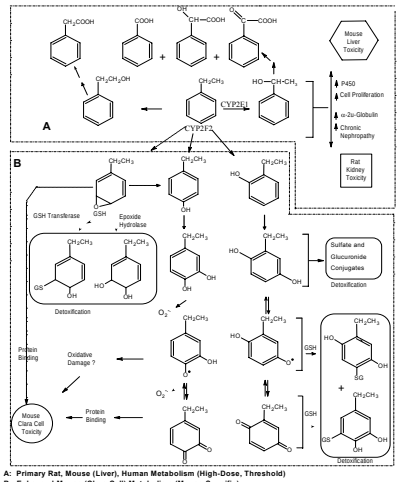
2

## Ethylbenzene Mode of Action Hypothesis

- Ethylbenzene toxicity parallels to structurally related compounds
  - Styrene
  - Naphthalene
  - Coumarin
- Mode of Action critically linked to metabolism
  - Rat kidney
  - Mouse liver
  - Mouse lung

3

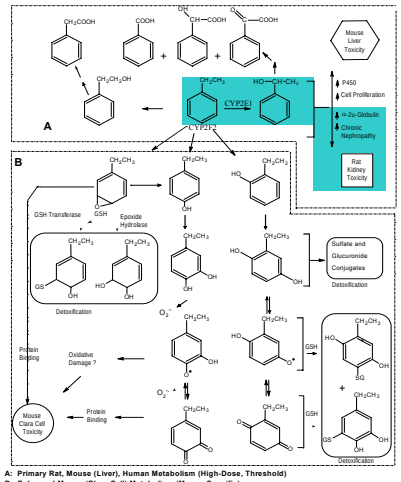
Postulated Mode of Action for Ethylbenzene




A: Primary Rat, Mouse (Liver), Human Metabolism (High-Dose, Threshold)
B: Enhanced Mouse (Clara Cell) Metabolism (Mouse-Specific)

4

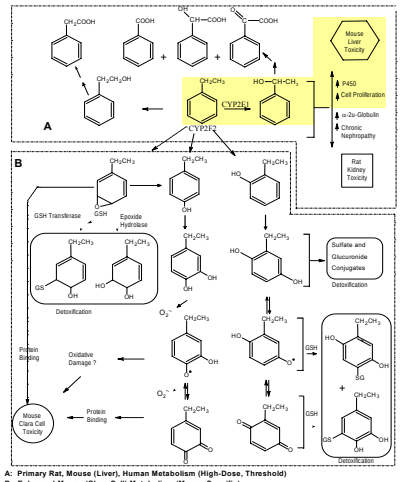
Postulated Mode of Action for Ethylbenzene




A: Primary Rat, Mouse (Liver), Human Metabolism (High-Dose, Threshold)
B: Enhanced Mouse (Clara Cell) Metabolism (Mouse-Specific)

5

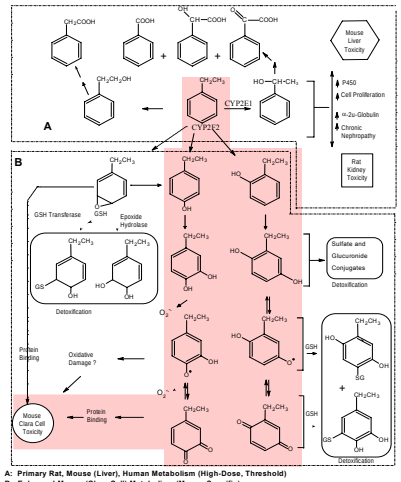
Postulated Mode of Action for Ethylbenzene




A: Primary Rat, Mouse (Liver), Human Metabolism (High-Dose, Threshold)
B: Enhanced Mouse (Clara Cell) Metabolism (Mouse-Specific)

6

Postulated Mode of Action for Ethylbenzene



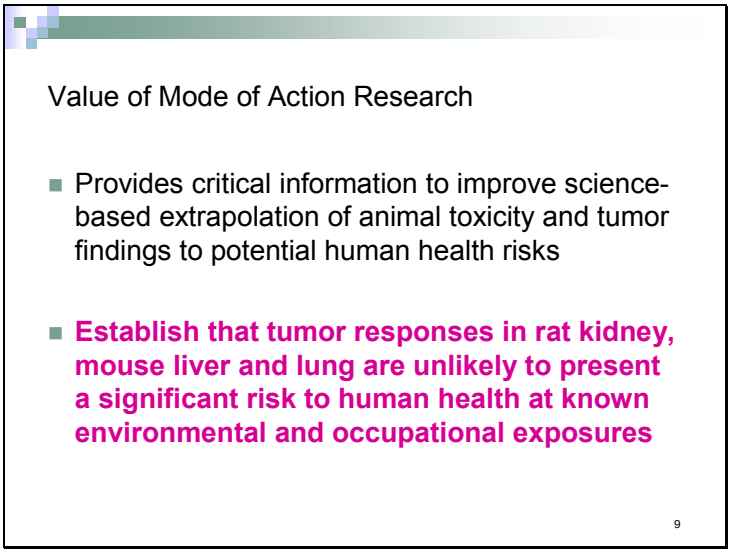

A: Primary Rat, Mouse (Liver), Human Metabolism (High-Dose, Threshold)
B: Enhanced Mouse (Clara Cell) Metabolism (Mouse-Specific)

7

## Male mouse lung MOA: Ongoing/planned research

- In vivo role of P450 2E1 and 2F2 in mediating ethylbenzene lung toxicity
  - impact of P450 inhibitors on terminal bronchiole toxicity and cell proliferation
  - impact of CYP2F2 knock-out/CYP knock-in animals
- Examine potential role of glutathione in modulating toxicity
  - ethylbenzene glutathione depletion in terminal bronchiole cells
    - immunohistochemical method to identify cell specific depletion
  - effect of glutathione depletion pre-treatment on ethylbenzene toxicity

8


Value of Mode of Action Research
Provides critical information to improve science-based extrapolation of animal toxicity and tumor findings to potential human health risks
Establish that tumor responses in rat kidney, mouse liver and lung are unlikely to present a significant risk to human health at known environmental and occupational exposures
9

American Chemistry Council

**American Chemistry Council Ethylbenzene Panel**

**VCCEP Peer Consultation for Ethylbenzene**

**Presentation on Risk Characterization**

**Lisa M. Sweeney, Ph.D., DABT**
**Michael L. Gargas, Ph.D.**
*The Sapphire Group, Inc.*

**February 23, 2007**

## Overview

- PBPK Modeling
- RfC Derivation
- RfD Derivation
- Cancer Reference Value Derivation
- Risk Characterization

2

## Uses of PBPK Models in EB VCCEP Assessment

- Exposure Assessment (VCCEP)
  - Modified version of human model used for estimation of lactational transfer of EB from mothers to infants
  - "Reality Check" for biomonitoring studies
- Used in experimental design
- Derivation of cancer and noncancer toxicity reference values based on internal dosimetry
  - Selection of "point of departure" for high-to-low dose extrapolation
  - Route-to-route extrapolation
  - Interspecies extrapolation

3

## PBPK Models for EB

- PBPK model for EB in the mouse was recently developed (Nong et al., 2007)
  - Updated since December VCCEP submission
- PBPK models of EB disposition previously developed for rat and human (Tardif et al., 1997; Haddad et al., 1999, 2000; Dennison et al., 2003)
- Rat model was extended for simulation of oral dosing and slightly modified to improve simulation of high concentrations

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## PBPK Models for EB

- Human model modified
  - Estimation of lactational transfer of EB from mothers to infants
  - Upper-bound estimate of formation of reactive metabolites in the lung
- Existing rat and human models were tested against additional data sets

5

## Results of Evaluation of PBPK Models for EB

- Rat
  - Modified Haddad/Krishnan model is appropriate for use simulation of repeated exposure of adult Sprague-Dawley and Wistar rats to EB by inhalation or oral route
- Mouse
  - Nong model is appropriate for simulation of repeated inhalation exposure of B6C3F1 mice to EB
- Human
  - Modified Haddad/Krishnan human model is appropriate for simulation of
    - Lactation dose to infants
    - Low-concentration exposures of adults
  - Sensitivity analysis of human model output indicates that default uncertainty factors are adequate for protection of children

6

## Toxicity Reference Value Derivation

- Dose-response analysis using BMDS was conducted based on PBPK-model derived internal doses
- Uncertainty Factors were applied to the point of departure
- The human-equivalent internal dose was transformed to the external Toxicity Reference Value using the PBPK model for route-to-route and interspecies extrapolation

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## RfC Derivation

- This presentation is limited to ototoxicity (rats) and liver effects (mice)
- Ototoxicity
  - Gagnaire et al. (2006)
    - Subchronic exposure of rats by inhalation
    - Increased outer-hair cell (OHC) loss at all tested concentrations (LOAEL = 200 ppm)
    - Relevant internal dose metric—AUC in richly perfused tissues
    - No subchronic-to-chronic UF needed
      - Composite UF = 30 proposed (UFA=3, UFH=10)

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## RfC Derivation

- Ototoxicity (cont'd)
  - Acceptable fit of rat dose-response data to the Hill Model
  - Point of departure was the 95% lower confidence limit on exceeding loss of 1.05% of outer hair cells in row 3
  - RfC for ototoxicity endpoint = 1 ppm

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## RfC Derivation

- Liver Effects: NTP (1999)
  - Liver syncitial alteration in chronically-exposed male mice (NOAEL = 75 ppm, LOAEL = 250 ppm)
  - Relevant internal dose metric: amount metabolized in the liver
  - Composite UF = 30 proposed (UFA=3, UFH=10)
  - Point of departure: lower confidence limit for 10% in increase in extra risk of syncitial alteration
  - Best fit was provided by the Gamma, Multistage, Q-Linear, and Weibull models
  - RfC for liver effects = 0.8 ppm

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## RfC Derivation

- An RfC of 0.8 ppm, based on liver effects in mice, is used for the VCCEP risk assessment

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## RfD Derivation

- Potential RfDs from previously discussed inhalation studies
  - Rat ototoxicity: 1.6 mg/kg/day
  - Mouse liver toxicity: 0.5 mg/kg/day

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## RfD Derivation

- Mellert et al. (2004, 2006)
  - Subchronic oral rat study
  - Liver and blood effects considered
  - Composite UF: 300 (UFS = 10, UFA = 3, UFH = 10)
  - Lowest potential RfD from this study = 0.2 mg/kg/day based on liver effects

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## RfD Derivation

- An RfD of 0.5 mg/kg/day is used
  - This value is derived from a chronic inhalation study rather than a subchronic oral study with the same target organ

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## Cancer Toxicity Reference Value Derivation

- Key study: NTP (1999) chronic bioassay results in rats and mice
- A threshold, “RfC-type” approach was selected based on the proposed modes of action for animal tumors (although MOAs indicate animal cancer of unlikely relevance to humans)

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## Cancer Toxicity Reference Value Derivation

- Female mouse liver tumors and male mouse lung tumors were evaluated
- Relevant internal dose metric: amount metabolized in tissue/tissue volume
- Cancer RfCs were derived based on 10% increase in tumor response

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## Cancer Toxicity Reference Value Derivation

- LEC10s determined for female mouse liver tumors and male mouse lung tumors
- Composite UF: 300 (UFL = 10, UFA = 3, UFH = 10)

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## Cancer Toxicity Reference Value Derivation

- Resultant reference values for cancer risk
  - Liver: 0.1 ppm; 0.07 mg/kg/d
  - Lung: 3.1 ppm; 4.3 mg/kg/d

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## Quantitation of Hazard

**Hazard Quotient (HQ):**

HQ = ADD/RV (*e.g.* RfD or RfC)

(If HQ $\leq$ 1.0, not considered a significant risk)

Where:

HQ = Hazard Quotient (unitless)

ADD = Average Daily Dose (mg/kg-d)

RV = Reference Value (mg/kg-d)

**Hazard Index (HI):**

$HI = \Sigma$ HQs

Sum contributions from various exposure pathways

(If total HI $\leq$ 1.0, not considered a significant risk)

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## VCCEP Total Hazard Indices For Most Highly Exposed Child

| Population Category | Exposure Category | Non-Cancer HI | Cancer HI |
|---|---|---|---|
| **Bottle-Fed Infant, Urban, Smoking Environment** | Central Tendency | 0.005 | 0.05 |
| **Breast-Fed Infant, Production Worker, Smoking Environment** | Upper Bound | 0.01 | 0.1 |

Indicates that even the most highly exposed child is not at risk from these ethylbenzene exposures

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## VCCEP Total Hazard Indices For Most Highly Exposed Prospective Parents

| Population Category | Exposure Category | Non-Cancer HI | Cancer HI |
|---|---|---|---|
| **Production Worker** | Central Tendency | 0.02 | 0.3 |
| | Upper Bound | 0.2 | 3 |

Indicates that the most highly exposed prospective parent has potential risk of liver cancer from these ethylbenzene exposures, but the actual risk is considered minimal to non-existent due to lack of relevance of mouse liver tumors to people.

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## Summary

- Data are adequate for risk assessment
- Animal toxicity observed $\geq$ 200 ppm for noncancer and 750 ppm for cancer
- MOAs indicate animal cancer of unlikely relevance to humans
- Human exposures are extremely low
- Children HI range = 0.005 - 0.05
- Prospective Parents HI range = 0.02 - 3

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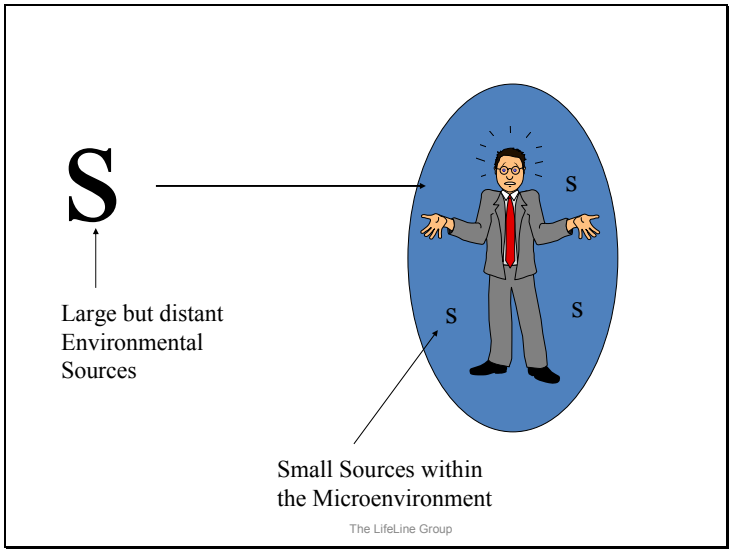
# Appendix D

# Panel member slide depicting person exposed from a large, distant environmental source and from multiple small near sources


S
Large but distant
Environmental
Sources
S
S
S
Small Sources within
the Microenvironment
The LifeLine Group

# Appendix E

# Voluntary Children's Chemical Evaluation Program (VCCEP)
# Peer Consultations on Ethylbenzene
# February 22-23, 2007

## Study Report from Dow Chemical Company:
## Ethylbenzene: In Vitro Metabolism with Rat, Mouse and Human Liver and Lung Microsomes – Phase II Study

## STUDY TITLE

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

### Test Guidelines

None

### Author(s)

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D. L. Rick, B.S.
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### Study Completion Date

20 February 2007

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### Performing Laboratory

Toxicology & Environmental Research and Consulting
The Dow Chemical Company
Midland, Michigan 48674

### Laboratory Project Study ID

051189

## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: ETHYLBENZENE

Title: ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

All phases of this study were conducted in compliance with the following Good Laboratory Practice Standards:

Japanese Ministry of International Trade and Industry (MITI)
GLP Standards Applied to Industrial Chemicals

US Environmental Protection Agency - TSCA GLPs
Title 40 CFR, Part 792 - Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards, Final Rule

Organisation for Economic Co-Operation and Development (OECD)
OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) EVN/MC/CHEM(98)17

European Community (EC)
European Parliament and Council Directive 2004/10/EC
(OJ No. L, 50/44, 20/02/2004)

Exception: Certificates of analysis of the test material and reference materials, (metabolites) as well as purity, were conducted by the supplier, no further GLP characterization was conducted.

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## QUALITY ASSURANCE STATEMENT

Compound: ETHYLBENZENE

Title: ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

This study was examined for conformance with Good Laboratory Practices as published by the USEPA TSCA; MITI, OECD, and EC The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 01 December 2005

| TYPE OF AUDIT: | DATE OF AUDIT: | DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT: |
|---|---|---|
| Final protocol | 15 November 2005 | 15 November 2005 |
| Study conduct | 21 September 2006 | 25 September 2006 |
| | 25 September 2006 | |
| Protocol, data, and draft report | 14 December 2006 | 19 December 2006 |
| Final report | The date of the signature below is the date of the final report audit. | |

The final report accurately reflects the raw data of the study.

P. A. Goodwin 2-20-2007

P. A. Goodwin, B.S., Auditor (Date)
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## SIGNATURE PAGE

Compound: ETHYLBENZENE

Title: ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

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## SUMMARY

Ethylbenzene (EB) is commonly used as an intermediate in the manufacture of styrene and synthetic rubber. It is also present in agricultural and home insecticide sprays, household degreasers, paints, adhesives, rust preventives and as a major component of mixed xylenes used as a solvent. Occupational exposure to EB may occur during the production of polystyrene as well as during production and use of mixed xylenes.

Ethylbenzene was incubated at concentrations ranging from 0.22 to 7 mM, with liver and lung microsomes of mouse, rat and human to measure the formation of 1-phenylethanol (1-PE), acetophenone, 2,5-ethylquinone, and 3,4-ethylquinone. The latter two reactive metabolites were monitored via a glutathione (GSH) trapping technique.

Molar conversion to the four metabolites varied quite broadly depending on microsome species/tissue and substrate concentration. None of the metabolites were formed at detectable levels in incubations with human lung microsomes. Alkyl-hydroxylated metabolites (1-phenylethanol, acetophenone) were formed at much higher levels than the ring-hydroxylated metabolites (catechols, hydroquinones, quinones). Molar conversion to the major metabolite, 1-PE, ranged from 1% (rat lung at 7mM EB) to 58% (mouse lung at 0.22 mM EB). This was equivalent to the formation of 0.09 µmole 1-PE by rat lung and 0.13 µmole by mouse lung; a difference of ~2-fold. The mass of 1-PE increased with increasing substrate levels, although the percent conversion (relative to starting substrate concentration) decreased. There was more 1-PE formed in mouse lung tissue incubations than in incubations with mouse liver microsomes. Levels of 1-PE formed in incubations with rat liver and lung microsomes were similar. The metabolism of EB to 1-PE, ranked according to species, was mouse > rat > human. 1-PE was formed at a level that was roughly an order of magnitude greater than acetophenone.

In a previous study in which EB was incubated with liver and lung microsomes (Saghir and Rick, 2005), very little aromatic-oxidation to either 2-ethylphenol (2EP) or 4-ethylphenol (4EP) was detected. It was surmised that the low levels of the mono-hydroxylated aromatic metabolites may have been due to further rapid oxidation to the di-hydroxylated catechol and quinone metabolites. It should be noted that in the earlier study, the GSH trapping technique used in this current study to afford greater sensitivity to quinones formed via catechols and hydroquinones was not employed. To investigate the potential for further oxidation, high concentrations of 2EP and 4EP were incubated

with microsomes, and the formation of ethylcatechol (ECat) and ethylhydroquinone (EHQ) monitored. Conversion from the mono- to the di-hydroxylated aromatics did occur, with molar conversion of 2EP to EHQ ranging from 6 to 9% in liver microsomes of the three species (mouse[8.9] > human[7.1] > rat[6.4]) and from 0.1 to 18% in lung microsomes (mouse[17.7] > rat[5.8] > human[0.1]). Conversion of 4EP to ECat ranged from 2 to 4% in liver microsomes (mouse[3.6] > human[2.1] ~ rat[2.0]) and from 0.3 to 7% in lung microsomes (mouse[7.1]>rat[1.4]>human[0.3]). In order to trap the reactive metabolites formed from 2EP and 4EP (i.e., the quinones derived from catechols and hydroquinones), experiments were conducted after adding excess GSH to each incubate.

Percent conversion of EB to ring-hydroxylated metabolites was much lower than what was observed for the alky-hydroxylated metabolites, ranging from 0.0001% (4EP-GSH; rat lung) to 0.6% (2EP-GSH; mouse lung). 2EP-GSH concentrations were typically 10-fold higher than 4EP-GSH. At lower substrate concentrations, more 2EP-GSH formed during incubations with lung microsomes than liver microsomes, for both rats and mice. More 2EP-GSH was formed in incubations with mouse liver microsomes than in incubations with liver microsomes from rat and human.

The highest levels of ring-hydroxylated metabolites were formed in incubations with mouse lung microsomes. Interestingly, both mouse and rat lung microsomes (and to a lesser extent, mouse liver microsomes) exhibited decreasing amounts of ring-oxidized metabolite formation with increasing concentrations of ethylbenzene. This suggests the possibility of cytochrome P450 suicide inhibition by reactive ring-oxidized metabolite(s). The possible suicide inhibition appears to be isozyme-specific in that generation of alkyl-oxidized metabolites was not similarly decreased with increasing EB substrate concentrations. This observation is consistent with the hypothesis that reactive ring-oxidized metabolites are likely formed by cytochrome P450 2F2, while alkyl-oxidized metabolite formation is mediated predominantly through cytochrome P450 2E1.

Although ring-oxidized metabolites accounted for a relatively small fraction of overall ethylbenzene metabolism, its selective elevation in mouse lung microsomes is nonetheless consistent with the hypothesized mode of action attributing preferential formation of lung-derived cytotoxic, ring-oxidized metabolites as driving the mouse lung specific toxicity of ethylbenzene.

## INTRODUCTION

Purpose

The objectives of this study were:

1. Determine the metabolism of 2- and 4-ethylphenol (metabolites of ethylbenzene) to catechols and hydroquinones by the lung and liver microsomes of three species.
2. Determine the rate of microsomal metabolism of ethylbenzene (EB) to 1-phenylethanol, catechols and hydroquinones

Test Guidelines

There are no established testing guidelines for this study.

Background

Ethylbenzene (EB) is commonly used as an intermediate in the manufacture of styrene and synthetic rubber. It is also present in agricultural and home insecticide sprays, household degreasers, paints, adhesives, rust preventives and as a major component of mixed xylenes used as a solvent. Occupational exposure to EB may occur during the production of polystyrene as well as during production and use of mixed xylenes (Fishbein, 1985).

No statistically significant increases in tumors were reported in Sprague-Dawley rats gavaged with 500 mg/kg/day EB (4 - 5 days/week, for 104 weeks) (Maltoni *et al.*, 1985). However, in an inhalation carcinogenicity study in which F344/N rats and B6C3F1 mice were exposed to 0, 75, 250, or 750 ppm EB 6 hours/day, 5 days/week, for 104 weeks, carcinogenic activity has been reported (NTP, 1999). Statistically identified neoplastic changes in the NTP (1999) study included: renal tubule adenomas in high exposure group male and female rats (changes to female kidney were noted only after step-sectioning), lung alveolar/bronchiolar adenomas in high exposure group male mice (and intermediate exposure group when combined with carcinomas), and liver adenomas in high exposure group female mice. A review of the genotoxicity of EB has concluded that the carcinogenicity of EB is likely attributable to a nongenotoxic mode of action (Henderson and Brusick, 2007).

The principle metabolic pathway of EB in humans and rats is alkyl-oxidation to 1-phenylethanol and subsequent alkyl-oxidized products (Engstrom, 1984; Charest-Tardif *et. al.*, 2006). In addition, Midorikawa *et.al.* (2004) demonstrated the potential for EB ring-oxidation to mono- and dihydroxylated metabolites, a pathway consistent with metabolism studies demonstrating similar metabolism of the structurally

analogous compound styrene to 4-vinylphenol (Bartels *et al.*, 2004). Formation of ring-oxidized metabolite(s) may be key to EB-induced mouse specific lung toxicity in that the mouse lung toxicity of styrene has also been linked to mouse-specific cytochrome P450 2F2 ring-oxidation metabolism (Cruzan, 2002). Consistent with that hypothesis, in a preliminary study (Saghir and Rick, 2005), ethylphenols (2- and 4-ethylphenol) were found as minor metabolites of EB. However, the preliminary study was not designed to examine if any ethylphenols formed might have been rapidly metabolized to downstream catechol/hydroquinone. Ethylphenols have been reported to cause direct pneumotoxicity in mice (Gelbke, personal communication, 2005). Since 2- and 4-ethylphenol have no structural alerts indicating cytotoxic potential, these findings point to the subsequent formation of ring-oxidized metabolites of ethylbenzene causing mouse specific lung toxicity.

Therefore, this study was designed to determine the potential of mouse, rat and human lung and liver microsomes to metabolize 2- and 4-ethylphenol to hydroquinone and catechol as well as rates of their formation from EB. Additionally, rates of the formation of 1-phenylethanol, acetophenone, and catechol/hydroquinone metabolites from EB were also determined from liver and lung microsomes of the three species.

Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan

Archiving

The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

Safety

Routine health and laboratory safety procedures were followed when handling all test materials, radiotracers, animals, and biological specimens. No other laboratory safety procedures were required.

## TEST MATERIAL INFORMATION

Test Material Name

Ethylbenzene; (EB); test substrate

Supplier, City, State (Lot/Reference Number)

EB: Sigma-Aldrich, St. Louis, MO (Lot No 01353MC)

Purity (Method of Analysis and Reference)

99.8% (analyzed by GC; vendor certificate of analysis)

Characteristics

*Molecular Formula*

$C_8H_{10}$

*Molecular Weight*

106.2

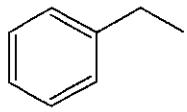
*Boiling and Melting Points*

b.p. = 136 °C; -95 °C

*Density*

0.867 g/mL

*Chemical Structure*



Metabolites

*1-Phenylethanol; (1-PE)*

Supplier/Lot Number

1-PE: Sigma-Aldrich (Fluka), St. Louis, MO (Lot No. 013398/1)

Purity

1-PE: 99.0% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

1-PE: $C_8H_{10}O$

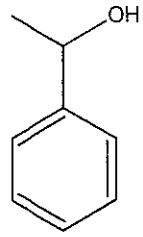
Molecular Weight

1-PE: 122.2

Density

1-PE: 1.012 g/mL

Chemical Structure (1-PE)




*Acetophenone; (AcPh)*

Supplier/Lot Number

AcPh: Sigma Sigma-Aldrich, St. Louis, MO (Lot No 07404KC)

Purity

AcPh: 99.5% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

AcPh: $C_8H_8O$

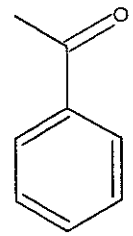
Molecular Weight

AcPh: 120.2

Density

AcPh: 1.03 g/mL

Chemical Structure (AcPh)




*2-Ethylphenol; (2EP)*

Supplier/Lot Number

2EP: Sigma-Aldrich, St. Louis, MO (Lot No. 15418DO)

Purity

2EP: 98.6% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

2EP: $C_8H_{10}O$

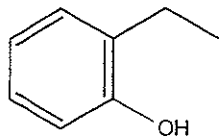
Molecular Weight

2EP: 122.2

Density

2EP: 1.037 g/mL

Chemical Structure (2EP)




*4-Ethylphenol, (4EP)*

Supplier/Lot Number

4EP: Sigma-Aldrich, St. Louis, MO (Lot No. 08805HO)

Purity

4EP: 98.5% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

4EP: $C_8H_{10}O$

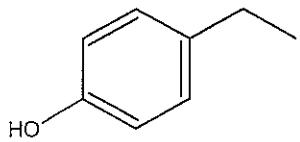
Molecular Weight

4EP: 122.2

Density

4EP: solid at room temperature

Chemical Structure (4EP)




*4-Ethylcatechol, (ECat)*

Synonym

3,4-dihydroxyethylbenzene (3,4-DiOHEB)

Supplier/Lot Number

ECat: Alfa Aesar (Lancaster), Ward Hill, MA (Lot No. 10020901)

Purity

ECat: 98.1% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

ECat: $C_8H_{10}O_2$

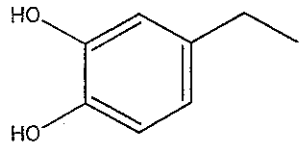
Molecular Weight

ECat: 138.2

Density

ECat: solid at room temperature

Chemical Structure (ECat)




*Ethylhydroquinone; (EHQ)*

Synonym

2,5-dihydroxyethylbenzene (2,5-DiOHEB)

Supplier/Lot Number

EHQ: Indofine, Hillsborough, NJ (Lot No. 94031)

Purity

EHQ: 98% (analyzed by titration; vendor certificate of analysis)

Molecular Formula

EHQ: $C_8H_{10}O_2$

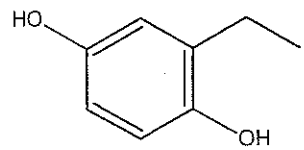
Molecular Weight

EHQ: 138.2

Density

EHQ: solid at room temperature

Chemical Structure (EHQ)



*4-Ethylresorcinol; (ERes)*

Synonym

2,4-dihydroxyethylbenzene

Supplier/Lot Number

ERes: Sigma-Aldrich, St. Louis, MO (Lot No. 08517LB)

Purity

ERes: 99.3% (analyzed by GC; vendor certificate of analysis)

Molecular Formula

ERes: $C_8H_{10}O_2$

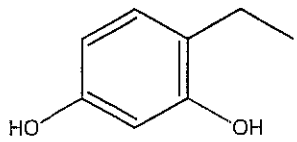
Molecular Weight

ERes: 138.2

Density

ERes: solid at room temperature

Chemical Structure (ERes)




Reagents.

Deuterated internal standards of EB, 1PE, AcPh, glutathione (GSH) and other reagents were purchased from Sigma-Aldrich Chemical (St. Louis, MO) unless otherwise noted, and all solvents were from VWR International (West Chester, PA) and were HPLC grade or better.

Synthesis of the GSH Conjugates of 2,5-Ethylquinone (2EP-GSH) and 3,4-Ethylquinone (4EP-GSH).

Standards of 2EP-GSH and 4EP-GSH were prepared from $^3$H-GSH (Perkin Elmer Life and Analytical Sciences, Boston, MA) and 2EP or 4EP. The incubation conditions employed for these preparations were as described in **Part 2: Rate of Microsomal Metabolism of EB to 1-Phenylethanol, Acetophenone, Ethylhydroquinone and Ethylcatechol**, below. Concentrations of either substrate were 1 mM, with 5 mM $^3$H-GSH (30 min incubations). Products in the incubation

solutions were characterized via LC-TOF/MS analysis (Fig 6, 7). Radiochemical analysis of the products eluting at 14-17 min from each incubation solution afforded concentrations of 19 and 14 ppm respectively, for 2EP-GSH and 4EP-GSH. These concentrations represent 5.1 and 3.7% conversion of the phenolic substrate to the final GSH-trapped products.

## TISSUE TYPES AND SOURCE/SUPPLIER

This study was conducted on microsomes made from liver and lung tissues obtained from male rats (un-induced Fischer-344), male mice (un-induced B6C3F1), and humans (mixed gender and race). Microsomes were obtained from XenoTech (Lenexa, Kansas) Rat liver and lung microsomes were prepared from the pool of 100 and 15 untreated animals, respectively Mouse liver and lung microsomes were prepared from the pool of 126 and 100 animals, respectively. Human liver microsomes were from the pool of 50 individuals of mixed gender, race (Caucasian, African, and Hispanic) and age (6 to 78 years old with most of them between 30 and 50 years), 31 of them died from cerebrovascular stroke, 12 from head trauma, 5 from anoxia, 1 from myocardial infarction, and 1 from aortic aneurysm. Human lung microsomes of non-smokers were prepared from the pool of 4 individuals of mixed gender (3males, 1 female), 1 died from stroke, 1 from interacranial hemorrhage, 1 from drug overdose, and 1 from motor vehicle accident

Microsomal samples were received in small aliquots (0.5 mL containing 5 or 10 mg protein) in eppendorf tubes on dry ice and immediately stored at –80 °C. Each microsomal preparation was analyzed by the supplier for at least CYP1A1/1A2 activity by the rate of metabolism of 7-ethoxyresorufin *O*-dealkylation (EROD assay). The samples were reanalyzed for CYP1A1/1A2 activity by EROD assay using a microplate fluorometric method (Kennedy and Jones, 1994). The 1A1/1A2 activity of human lung microsomes was about 7% of the levels measured in rat lung microsomes, similar to what has been reported by Keith *et al* (1987) and Devereux *et al* (1989), and was 4-fold lower than what has been reported for nonsmoker human lung microsomes prepared from fresh tissues obtained within 15 minutes of lobectomy (Smith *et al*, 2001). The 1A1/1A2 activity of mouse lung microsomes was 16-fold higher than rat lung The 1A1/1A2 activity in the liver microsomes was in the order of mouse > rat >> human (Text Table 1)

Text Table 1. EROD Activity of Mouse, Rat and Human Liver and Lung Microsomes

| | *EROD Activity (pmol/mg protein/min)* | | |
|---|---|---|---|
| Tissue | Mouse | Rat | Human |
| Liver | 2720 ± 80 | 2680 ± 0 | 51.5 ± 1.0 |
| Lung | 374 ± 1.0 | 2.4 ± 0.02 | <0.2 |

## STUDY DESIGN

Part 1: Microsomal Metabolism of 2- and 4-Ethylphenol

One concentration of 2- or 4-ethylphenol (1 mM final concentration) was incubated for 30 minutes at 37 °C with mouse, rat and human lung and liver microsomes (1 mg protein/mL 0 1 M phosphate buffer, cofactors, pH 7.4) in 24-mL gas-tight glass vials. These levels were, at a minimum, 100-fold greater than the concentrations of the metabolites detected via microsomal incubations with EB in the preliminary study; thus, these levels were selected to provide enough metabolite(s) for analytical quantification. Each treatment (substrate/tissue type) was comprised of three replicates. Two types of control incubations were also conducted. One control type contained all components (0.1M phosphate buffer, NADPH, microsomes), but no substrate; the second control was conducted with substrate and microsomes, but no NADPH. After the completion of the incubations, samples were analyzed for the loss of 2- and 4-ethylphenol, as well as formation of ethylhydroquinone and ethylcatechol by high performance liquid chromatography with ultraviolet detection (HPLC/UV).

As a basis of comparison with the preliminary EB metabolism study (Saghir and Rick, 2005), mouse liver and/or lung microsomes were incubated with 750 µg of EB delivered to 1 mL of the incubation medium using propylene glycol (PG) as vehicle (or, 7 mM EB in test system). This was equivalent to the amount of EB delivered to the *in vitro* incubation systems of the preliminary study that were prepared with 7500 ppm of EB vapor in the (23-mL) headspace of the incubation vials (Saghir and Rick, 2005). After completion of 30 minute incubations with EB, samples from the current study were analyzed for formation of 1-phenylethanol, 2EP, 4EP (as done in the preliminary study), as well as ethylhydroquinone (EHQ) and ethylcatechol (ECat). No attempt was made to measure EHQ and ECat in the preliminary study since detection of those metabolites was not amenable to the gas chromatographic technique employed. However, the HPLC/UV analysis employed in the current study afforded a better option to detect those metabolites (if formed). The principal rationale in the attempts to monitor the formation of EHQ and ECat in the present study was that 2EP and 4EP were detected at only very trace amounts in the earlier study (molar conversion of EB to 2EP<0.3%; conversion of EB to 4EP<0.02%). It was surmised that if 2EP or 4EP had been formed, they may not have been detected if these metabolites were transient and further metabolized to EHQ and ECat.

Part 2: Rate of Microsomal Metabolism of EB to 1-Phenylethanol, Acetophenone, Ethylhydroquinone and Ethylcatechol

Prior to the definitive incubations of EB with liver and lung microsomes of three species, to be described later, probe incubations of EB with mouse liver microsomes were conducted (at a single substrate concentration) to assess the linearity of the rates of formation of 1-phenylethanol, acetophenone, 2,5-ethylquinone, and 3,4-ethylquinone over time (i.e., assess optimal incubation time used for the definitive kinetics experiments). Mouse liver microsomes were incubated with 2 mM EB (and 1 mg protein/mL 0.1 M phosphate buffer, pH 7.4, 1 mM NADPH, 10 mM glutathione) in glass vials. Incubations were conducted for 10, 15, 30, 60, and 90 minutes (two replicate vessels per incubation time). Note that except for the differences in substrate concentrations, incubation solutions of the definitive kinetic experiments were prepared with the same components as the time-course incubations. EB was introduced to the incubation vessels using propylene glycol as the vehicle; 10 µL of the PG-based dosing solution was added to each (1 mL) incubation solution (thus, PG comprised ~1% of the final solution).

Following the completion of the incubations to assess optimal incubation time, the definitive Part 2 experiment to determine kinetics of 1-PE and AcPh (major metabolites of EB) as well as kinetics of 2,5- and 3,4-ethylquinone (potential reactive metabolites of EB) formation were conducted. Liver and lung microsomes of three species (rat, mouse, human) were incubated in triplicate at 37 °C with EB at six concentrations (Text Table 2). The substrate concentrations were 7.0, 3.5, 1.8, 0.9, 0.45, and 0.22 mM EB. The incubation time was 30 min, based on the results of the time-course experiments. Six separate PG-based solutions of EB were prepared such that the EB, for all treatment levels, was delivered in a volume of 10 µL.

Text Table 2. Number of Incubations/Substrate Concentration

| | Tissue Type/Replicates | | | |
|---|---|---|---|---|
| Species | Liver | Lung | EB Conc. | Total[a] |
| Mouse | 3 | 3 | 6 | 36 |
| Rat | 3 | 3 | 6 | 36 |
| Human | 3 | 3 | 6 | 36 |

[a]Total samples : 108 samples, not including controls.

Three types of control incubations were conducted. Control Type I contained all components (0.1 M phosphate buffer, NADPH, microsomes, glutathione, 10 µL PG), but no EB; Control Type II was conducted with incubates containing 0.45, 1.8, or 7.0

mM EB (single replicate for each [of six] microsome types), glutathione (GSH) and microsomes, but no NADPH; Control Type III contained all components (with 0 45 mM EB), but no microsomes.

Following the incubation, each solution was processed (to be described in the next paragraph) for two types of analysis. The first analysis, by gas chromatography with mass spectrometry (GC/MS), was conducted for the determination of EB and the major (volatile) metabolites, 1-PE and AcPh. The second analysis utilized a glutathione trapping technique (which is the reason that 10 mM GSH was added to the incubation solutions) as previously employed for the *in vitro* metabolism of 4-vinylphenol to hydroquinone and catechol (Bartels *et al.*, 2004). The GSH conjugates were analyzed by high performance liquid chromatography with multiple reaction monitoring mass spectrometry (HPLC/MRM/MS). The rationale behind that technique is that reactive metabolites of EB (e.g., quinones of ethylhydroquinone or ethylcatechol), if formed, will react with the excess GSH to form the glutathione conjugates. The structures of the GSH conjugates of the reactive quinones are shown in Figure 1. For purposes of this report, those conjugates will be referred to as 2EP-GSH and 4EP-GSH. Briefly, the pathways for formation can be summarized as:

a) EB → 2EP → ethylhydroquinone → 2,5-ethylquinone (+GSH) → **2EP-GSH**

b) EB → 4EP → ethylcatechol → 3,4-ethylquinone (+GSH) → **4EP-GSH**

Following the 30 min incubations, 0.2 mL of a so-called "kill solution" was injected through the septum of each incubation vial (i.e., vials remained sealed from the point of mixing components through incubation period, and until after kill solution was added). The kill solution was comprised of 2% formic acid (to reduce pH), 5% ascorbic acid (anti-oxidant), and a mixture of internal standards (described next) in a solution of 20/80 $H_2O$/acetonitrile. In addition to controlling pH and reducing potential for oxidation, the kill solution served the following purposes: a) stop the metabolic reactions; b) enhance the solubility of EB prior to analysis due to the addition of acetonitrile (as substrate concentrations in treatments of 1.8 mM and above may have exceeded the water solubility of EB), c) and internal standards of volatile analytes would control for the loss those analytes during sample preparation in addition to controlling for the variability in MS response during analysis. The internal standards contained in the kill solution were $d_{10}$-EB, $d_5$-1-PE, and $d_5$-AcPh (with "$d_x$" signifying the number of deuteriums substituted in the molecular structure), at a concentration of 100 µg/mL, each. After the addition of the kill solution, the vials were briefly chilled by placing them in an ice bath. Then the vials were opened, and to remove the precipitated protein and other solids, the samples were filtered through

Whatman (Florham Park, New Jersey) 13-mm ZC PTFE syringe filters (0.2 µ pore; Cat# 6844-1302) and collected in autosampler vials. The syringes used were B-D (Franklin Lakes, New Jersey) 1-mL plastic body; PN 309628

The collected filtrate was subdivided for the two separate assays, with each preparation completed as follows. For GC/MS analysis of EB, 1-PE, and AcPh, an 0.3-mL aliquot was transferred to a 1-dram vial and extracted with 0.6 mL of $CS_2$ (by shaking for 30 min on a flatbed shaker). The bottom layer ($CS_2$) was transferred to a 1-dram vial containing ~100 mg $MgSO_4$ to remove absorbed water from the $CS_2$. The "dried" $CS_2$ was then decanted to an autosampler vial for analysis by GC/MS.

In the preparation for analysis of reactive metabolites (e.g., 2EP-GSH and 4EP-GSH), a 0.3-mL aliquot of the initial filtrate was transferred to an autosampler vial to which was added a laboratory-synthesized internal standard ($d_4$-4EP-GSH).

Samples were stored at –80 °C while awaiting analysis.

## DATA ANALYSIS

Descriptive statistics (*i.e.*, mean ± standard deviation) for the depletion of parent EB and formation of metabolites were calculated using Microsoft Excel® spreadsheets in full precision mode (15 digits of accuracy). The rates of metabolism were calculated using standard methods (e.g., Eadie-Hofstee, Lineweaver-Burk plots). Details of the statistical methods employed are included in the study file.

## RESULTS AND DISCUSSION

Microsomal Metabolism of 2- and 4-Ethylphenol to Dihydroxyethylbenzenes

The results from the microsomal incubations of 2- and 4-ethylphenol that were conducted to determine conversion of those substrates to the ring-hydroxylated metabolites ethylhydroquinone and ethylcatechol are shown in Tables 1 and 2, respectively. In general a higher percent conversion was observed in the metabolism of 2EP to EHQ compared to the conversion of 4EP to ECat. The highest conversion occurred in incubations with mouse lung microsomes for both metabolic pathways; approximately 18% of the 2EP was metabolized to EHQ and ~7% of the 4EP was metabolized to ECat. Likewise, in incubations with liver microsomes, the highest percent conversion occurred in incubations with mouse liver microsomes. Compared across tissue types, the percent conversion from mouse lung incubations was roughly 2-fold greater than the conversion obtained from incubations with mouse liver microsomes. The percent conversion (of both metabolic pathways) was approximately equivalent from incubations with rat lung and liver tissues. Human

liver microsomes converted more of the ethylphenols to the corresponding di-hydroxyethylbenzene than the lung microsomes. Between the two pathways, human liver microsomes formed about 3-fold higher EHQ than ECat.

To summarize this series of experiments, molar conversion of 2EP to EHQ ranged from 6 to 9% in liver microsomes of the three species (mouse [8.9] > human [7.1] > rat [6.4]) and from 0.1 to 18% in lung microsomes (mouse [17.7] > rat [5.8] > human [0.1]). Conversion of 4EP to ECat ranged from 2 to 4% in liver microsomes (mouse [3.6] > human [2.1] ~ rat [2.0]) and from 0.3 to 7% in lung microsomes (mouse [7.1] > rat [1.4] > human [0.3]).

Microsomal Metabolism of Ethylbenzene to Dihydroxyethylbenzenes

Because of the relatively high conversions of ethylphenols to dihydroxyethylbenzenes that were attained in the incubations just discussed (although the starting ethylphenol concentrations were at least 100-fold higher than the levels detected in incubations with EB from the preliminary study), the next series of experiments involved the incubation of 7 mM EB with liver and lung microsomes of the three species to monitor formation of hydroquinone and catechol. This was intended to determine whether the low conversion of EB to ethylphenols observed in the preliminary experiments were due to the transient nature of those metabolites (that were rapidly metabolized to dihydroxyethylbenzenes). Neither EHQ nor ECat was formed in any of the incubations (with mouse, rat, human; liver or lung microsomes) at levels exceeding the limit of detection (0.001 µmole per incubation vessel; corresponding to ~0.01% conversion; data not shown). The failure to detect EHQ and ECat does not mean that they were not formed in the incubations with EB. It is likely that such metabolites were formed, but then underwent further oxidation to quinones that could not be detected without using the GSH-trapping technique (employed in other incubations of this study).

Although the primary objective of the incubations just discussed was to determine conversion of EB to hydroquinones and catechols, a secondary objective was to measure levels of 1-PE, 2EP, and 4EP and to compare those levels (from incubations in which EB was introduced using PG vehicle) with the levels of those metabolites measured in the preliminary study, in which EB was introduced as a vapor into the incubation vessels. The average percent conversions of EB to 1-PE in the experiments of this study (at a 7mM EB concentration) were 2.4%, 2.0% and 0.9% in liver microsomal incubates of mouse, rat, and human, respectively (data not shown). These values are in good agreement with the percent conversions of EB to 1-PE (in the preliminary study, with EB dosed at 7500 ppm in headspace) observed in liver microsome incubations of the same three species, specifically: 2.7%, 1.7%, and 1.6%.

The average percent conversions of EB to 1-PE in the incubations (of current study) with lung microsomes (of mouse and rat) were 6.1% and 1.7%, respectively. In comparison, conversions of 7500 ppm EB to 1-PE in incubations with lung microsomes (of preliminary study) were 5.2% and 1.6%, respectively. 1-PE was not detected in incubations of EB with human lung microsomes in either the preliminary or the current study (limits of detection ~0.0005 µmoles per vessel in preliminary study; ~0.001 µmoles per vessel in current study). 4EP was not detected in any of the incubations with EB introduced in PG vehicle (current study). In contrast, 2EP was formed at detectable levels, but only in incubations with mouse lung microsomes (of current study). The percent conversion of EB to 2EP in the current study was approximately 0.1%, which was the same percent conversion of EB to 2EP obtained in mouse lung incubations from the preliminary study in which EB was introduced as a vapor.

Probe Incubations to Assess Linearity of Metabolite Formation Over Time

The results from the time-course experiments, in which 2 mM EB was incubated with mouse liver microsomes at various incubation times, are presented in Tables 3 and 4 (with accompanying graphs). These experiments were designed to monitor the time course of conversion of EB to 1-PE, AcPh, EHQ, and ECat. The results of these experiments indicated that the rate of formation of all four metabolites was linear for at least 30 minutes. Therefore, a 30-minute incubation period was used in the *in vitro* kinetic experiments.

Comparative Levels of Metabolite Formation

Table 5 summarizes the percent conversion of EB to the four metabolites of interest (1-phenylethanol, acetophenone, and the glutathione-trapped conjugates of 2,5- and 3,4-ethylquinone) formed during 30-minute incubations with liver microsomes of mouse, rat, and human. Table 6 shows the percent conversion of EB to those same four metabolites following incubations with lung microsomes. Individual data are organized and presented in four appendix tables. Appendix Table 1 contains data from the GC/MS analysis of liver microsome incubates for EB and its primary (volatile) metabolites, 1-PE and AcPh. Appendix Table 2 contains individual data from the analysis of lung microsome incubation solutions for EB, 1-PE and AcPh. Appendix Table 3 contains data from the analysis of liver microsome incubates for the glutathione-trapped conjugates of reactive quinones (formed via oxidation of ethylhydroquinone and ethylcatechol; abbreviated as 2EP-GSH and 4EP-GSH, respectively). Appendix Table 4 contains data from the analysis of lung microsome incubates for 2EP-GSH and 4EP-GSH.

As shown in Tables 5 and 6, incubations of EB with human lung microsomes failed to produce detectable levels of any of the four metabolites. 1-PE represents the major metabolite formed during microsomal incubations with EB, regardless of species or tissue type. This is consistent with the results from the preliminary study of Saghir and Rick (2005) as well as the results reported by Engstrom (1984) and Midorikawa et al (2004). Except for incubations with human lung microsomes, 1-PE was formed at detectable levels in all in vitro incubations. Note that analyte concentrations in the appendices are reported in units of µg/mL (for EB, 1-PE, and AcPh) and ng/mL (for 2EP-GSH and 4EP-GSH), which are somewhat easier to review than the values expressed as µmoles in Tables 5 and 6. Levels of 1-PE increased with increasing substrate concentrations for all species/microsome tissue types (although percent conversion values decreased). There was more 1-PE formed in mouse lung tissue incubations than in incubations with mouse liver microsomes, but 1-PE formed in incubations with rat liver and lung microsomes were comparable. 1-PE levels were 2 to 5-fold higher in incubates of mouse lung compared to levels in rat lung incubation solutions. Levels of 1-PE formed in the liver microsome incubates followed the general rank order of mouse > rat > human. The greatest disparity in 1-PE formation was observed at lowest substrate concentrations. 1-PE plateaued at similar levels during incubations with mouse and rat liver microsomes which is an indication that the primary route of EB metabolism (e.g., alkyl-oxidation) in mouse and rat microsomal systems was saturated at the highest substrate concentrations. 1-PE levels formed in human liver incubations remained lower than those of the other two species (at highest substrate level).

Acetophenone was formed at much lower levels than 1-PE (it was not detectable in the majority of samples from the rat lung and human liver incubations), but otherwise followed trends similar to those observed for 1-PE. In general, AcPh levels were at least 10-fold lower than 1-PE levels when making comparisons within a particular species/tissue type; the disparity between 1-PE and AcPh levels tended to increase with increasing substrate concentrations. As observed for 1-PE, the highest amount of AcPh was formed in incubations with mouse lung microsomes. Levels in mouse liver incubations were higher than levels in rat liver incubations at low substrate concentrations, but were similar at the highest substrate levels. AcPh levels generally increased with increasing substrate concentrations, but not to the extent that was observed for 1-PE (most pronounced in incubations with rat liver microsomes). Contrary to the general trend, more AcPh was formed in incubations with rat liver microsomes than in incubations with rat lung microsomes.

Representative chromatograms from the GC/MS analyses of incubation solutions for EB, 1-PE, and AcPh are shown in Figures 2, 3, and 4, respectively

Glutathione adducts of the reactive metabolites (i e., quinones of ethylhydroquinone and ethylcatechol) were formed at much lower levels than that of 1-PE and AcPh Molar conversions ranged from 0.0001% (4EP-GSH; rat lung) to 0.6% (2EP-GSH; mouse lung) compared to conversions of EB to 1-PE that ranged from 1% (rat lung; 7mM EB) to 58% (mouse lung; 0.22 mM EB). The GSH adducts of 2,5-ethylquinone were generally formed in higher amounts than GSH adducts of 3,4-ethylquinone; typically the differences were at least 10-fold

The highest mass of 2EP-GSH formed (not simply highest percent conversion) occurred in incubations of EB with mouse lung microsomes at the lowest substrate concentrations. At lower substrate concentrations, there was more 2EP-GSH formed during incubations with lung microsomes than in incubations with liver microsomes (of a given species; most pronounced for mouse lung vs mouse liver). For example, $1.2 \times 10^{-3}$ µmoles of 2EP-GSH were formed in 0.22 mM EB incubations with mouse lung microsomes compared to $6.3 \times 10^{-4}$ µmoles formed in incubations with mouse liver microsomes Similarly, for incubations with microsomes from rat tissues, $1.3 \times 10^{-4}$ µmoles of 2EP-GSH were formed in 0.22 mM EB incubations with rat lung microsomes compared to $6.0 \times 10^{-5}$ µmoles formed in incubations with rat liver microsomes. This relationship was reversed at the higher substrate concentrations because the mass amounts of 2EP-GSH formed in incubations with lung microsomes (of both mouse and rat) decreased with increasing substrate levels, whereas 2EP-GSH levels in incubations with rat liver microsomes increased (in a more conventional manner) as substrate levels increased. Interestingly, mass amounts of 2EP-GSH formed in incubations with mouse liver microsomes also decreased as substrate levels increased, but not to the extent as observed in mouse lung incubations.

There was more of the 2EP-GSH adducts formed in incubations with mouse liver microsomes than in incubations with rat and human liver microsomes; typically the difference was an order of magnitude and was most pronounced at the lower substrate concentrations. Similarly, more 4EP-GSH was formed in mouse liver incubations than in rat liver incubations, but the differences ranged from about 4-fold at the lowest substrate concentration to approximately equivalent at the highest EB concentration. One interesting exception was that 4EP-GSH formation was greater in incubations with human liver microsomes than in incubations with mouse or rat liver microsomes.

A representative chromatogram from the HPLC/MRM/MS analyses of incubation solutions for 2EP-GSH and 4EP-GSH is shown in Figure 5. Typical time-of-flight mass spectra of 2EP-GSH and 4EP-GSH are shown in Figures 6 and 7.

Kinetics of Metabolite Formation

Lineweaver-Burk plots were constructed to derive $K_m$ and $V_{max}$ values to describe conversion of EB to the major metabolites 1-PE and AcPh, and when possible conversion to the reactive quinones. Derivation of $K_m$ and $V_{max}$ values was not possible for metabolites whose rates of formation decreased as substrate concentrations increased (e.g., formation of 2EP-GSH in incubations of EB with mouse lung microsomes). The $K_m/V_{max}$ values are summarized in Table 7 for those compounds whose rates of formation increased with increasing substrate concentrations. A representative graph (and Lineweaver-Burk plot) showing the rates of formation of 1-PE and acetophenone in incubations with lung microsomes is shown in Figure 8. A representative graph (and Lineweaver-Burk plot) showing the rates of formation of 2EP-GSH in incubations with liver microsomes is shown in Figure 9.

As previously mentioned, $K_m/V_{max}$ values could not be derived for metabolites whose rates of formation decreased with increasing substrate levels. However, to provide some information as to the velocities of conversion (µmoles/mg protein/min), velocities were calculated at each substrate concentration for those metabolites whose rates of formation decreased as substrate concentrations increased. Those maximum velocities (typically occurring at the 0.22 mM EB concentration) and minimum velocities (typically occurring at the 7 mM EB concentration) are presented in Table 8.

## CONCLUSIONS

Ethylbenzene was incubated at concentrations ranging from 0.22 to 7 mM, with liver and lung microsomes of mouse, rat and human to measure the formation of 1-phenylethanol, acetophenone, 2,5-ethylquinone, and 3,4-ethylquinone. The latter two reactive metabolites were monitored via a glutathione trapping technique.

Molar conversion to the four metabolites varied quite broadly depending on microsome species/tissue and substrate concentration. None of the metabolites were formed at detectable levels in incubations with human lung microsomes. Alkyl-hydroxylated metabolites (1-PE, acetophenone) were formed at much higher levels than the ring-hydroxylated metabolites (catechols, hydroquinones, quinones). Molar conversion to the major metabolite, 1-PE, ranged from 1% (rat lung at 7mM EB) to 58% (mouse lung at 0.22 mM EB). This was equivalent to the formation of 0.09 µmole 1-PE by rat lung and 0.13 µmole by mouse lung; a difference of ~2-fold. The

mass of 1-PE increased with increasing substrate levels, although the percent conversion (relative to starting substrate concentration) decreased. There was more 1-PE formed in mouse lung tissue incubations than in incubations with mouse liver microsomes. Levels of 1-PE formed in incubations with rat liver and lung microsomes were similar. The metabolism of EB to 1-PE, ranked according to species, was mouse > rat > human. 1-PE was formed at a level that was roughly an order of magnitude greater than acetophenone.

In a previous study in which EB was incubated with liver and lung microsomes, very little aromatic-oxidation to either 2-ethylphenol (2EP) or 4-ethylphenol (4EP) was detected (GSH-trapping technique was not employed). It was surmised that the low levels of the mono-hydroxylated aromatic metabolites may have been due to further rapid oxidation to the di-hydroxylated catechol and quinone metabolites. To investigate the potential for further oxidation, high concentrations of 2EP and 4EP were incubated with microsomes, and the formation of ethylcatechol and ethylhydroquinone monitored. Conversion from the mono- to the di-hydroxylated aromatics did occur, with molar conversion of 2EP to EHQ ranging from 6 to 9% in liver microsomes of the three species (mouse[8.9] > human[7.1] > rat[6.4]) and from 0.1 to 18% in lung microsomes (mouse[17.7] > rat[5.8] > human[0.1]). Conversion of 4EP to ECat ranged from 2 to 4% in liver microsomes (mouse[3.6] > human[2.1] ~ rat[2.0]) and from 0.3 to 7% in lung microsomes (mouse[7.1]>rat[1.4]>human[0.3]). In order to trap the reactive metabolites formed from 2EP and 4EP (i.e., quinones derived from catechols and hydroquinones), experiments were conducted after adding excess GSH to each incubate.

Percent conversion of EB to ring-hydroxylated metabolites was much lower than what was observed for the alky-hydroxylated metabolites, ranging from 0.0001% (4EP-GSH; rat lung) to 0.6% (2EP-GSH; mouse lung). 2EP-GSH concentrations were typically 10-fold higher than 4EP-GSH. At lower substrate concentrations, more 2EP-GSH formed during incubations with lung microsomes than liver microsomes, for both rats and mice. More 2EP-GSH was formed in incubations with mouse liver microsomes than in incubations with liver microsomes from rat and human.

The highest levels of ring-hydroxylated metabolites were formed in incubations with mouse lung microsomes. Interestingly, both mouse and rat lung microsomes (and to a lesser extent, mouse liver microsomes) exhibited decreasing amounts of ring-oxidized metabolite formation with increasing concentrations of ethylbenzene. This suggests the possibility of cytochrome P450 suicide inhibition by reactive ring-oxidized metabolite(s). The possible suicide inhibition appears to be isozyme-specific in that

generation of alkyl-oxidized metabolites was not similarly decreased with increasing EB substrate concentrations. This observation is consistent with the hypothesis that reactive ring-oxidized metabolites are likely formed by cytochrome P450 2F2, while alkyl-oxidized metabolite formation is mediated predominantly through cytochrome P450 2E1.

Although ring-oxidized metabolites accounted for a relatively small fraction of overall ethylbenzene metabolism, its selective elevation in mouse lung microsomes is nonetheless consistent with the hypothesized mode of action attributing preferential formation of lung-derived cytotoxic, ring-oxidized metabolites as driving the mouse lung specific toxicity of ethylbenzene.

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Figure 1. Reaction of 2,5-Ethylquinone and 3,4-Ethylquinone with GSH to form the Conjugates Analyzed by HPLC/MRM/MS (conjugates abbreviated as 2EP-GSH and 4EP-GSH in report)

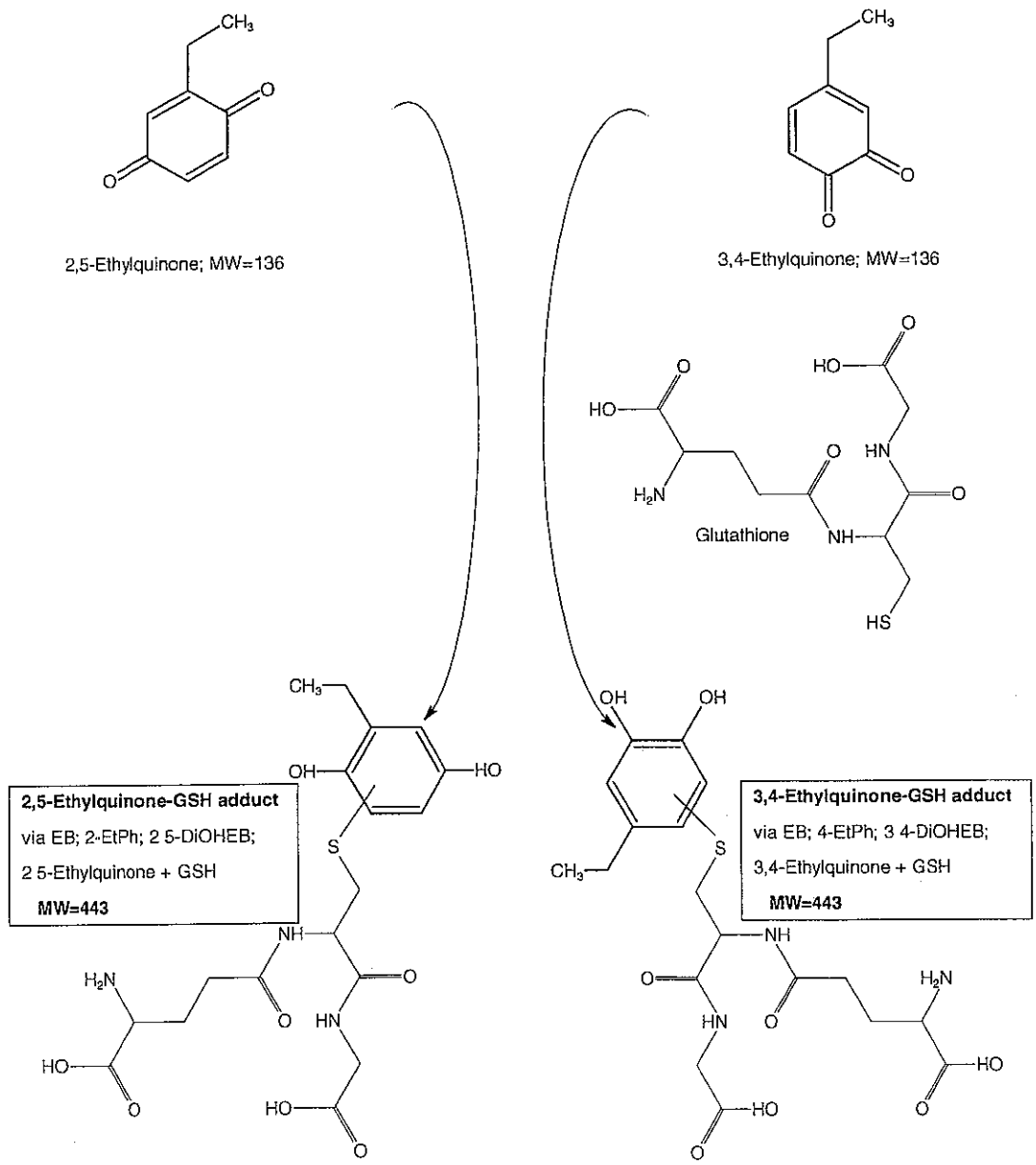


Figure 2 Representative GC/EI-MSD Chromatogram of a Mouse Lung Incubation Sample Extract Analyzed for Ethylbenzene (m/z 106; 131 µg/mL) and $d_{10}$-Ethylbenzene (m/z 116, internal standard; 100 µg/mL)

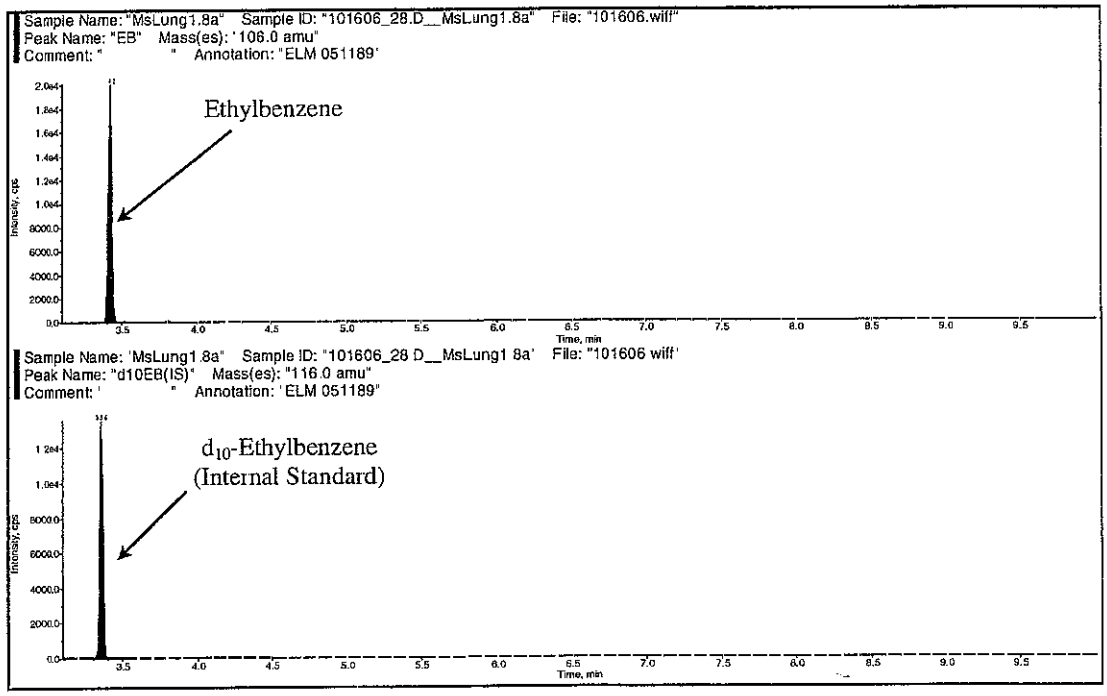


Figure 3 Representative GC/EI-MSD Chromatogram of a Mouse Lung Incubation Sample Extract Analyzed for 1-Phenylethanol (m/z 122; 53 6 µg/mL) and $d_5$-1-Phenylethanol (m/z 127, internal standard; 100 µg/mL)

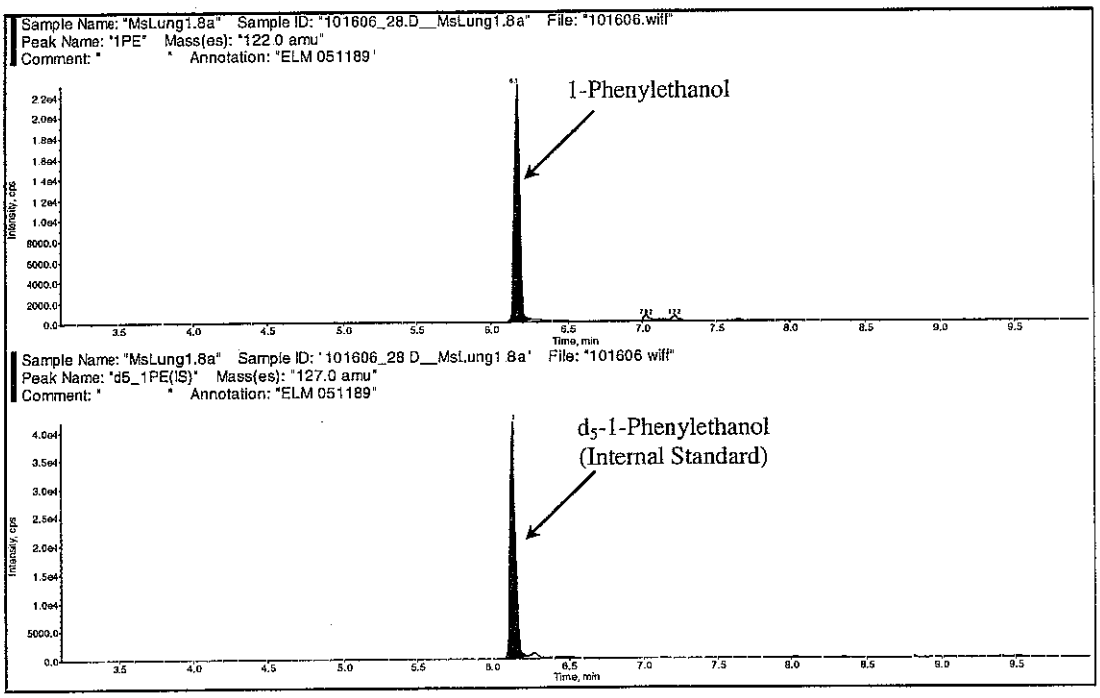


Figure 4 Representative GC/EI-MSD Chromatogram of a Mouse Lung Incubation Sample Extract Analyzed for Acetophenone (m/z 120; 2.51 µg/mL) and $d_5$-Acetophenone (m/z 125, internal standard; 100 µg/mL).

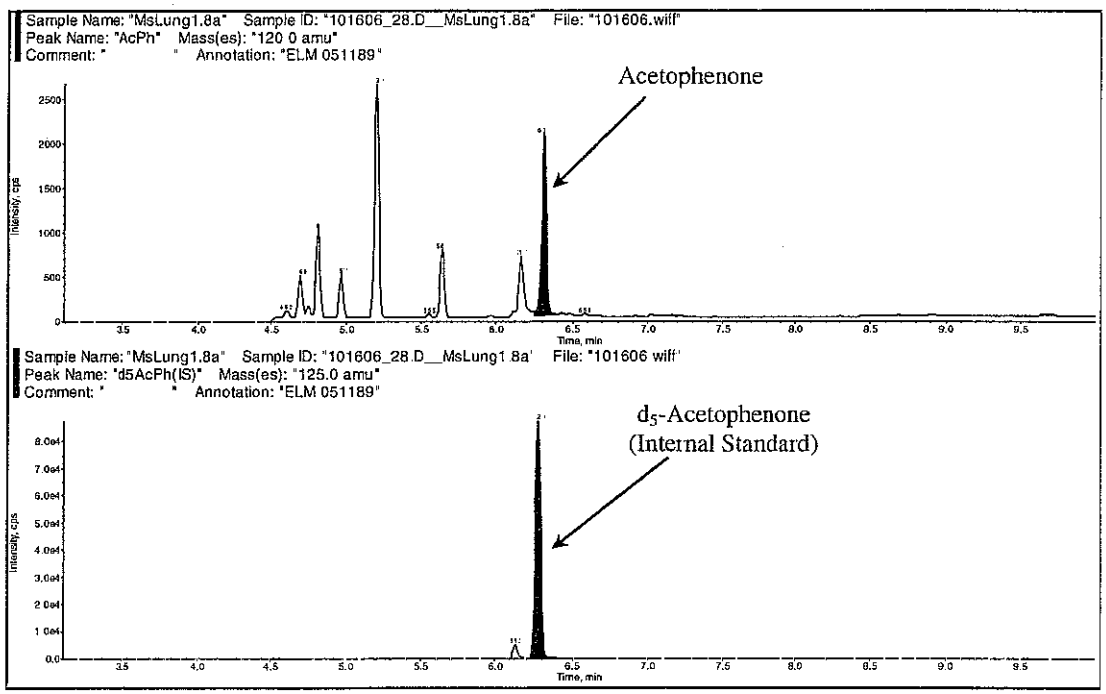


Figure 5 Representative HPLC/MRM/MS Chromatogram from Incubation of 2EP-GSH and 4EP-GSH isomers, arising from EB with Mouse Lung Microsomes and GSH

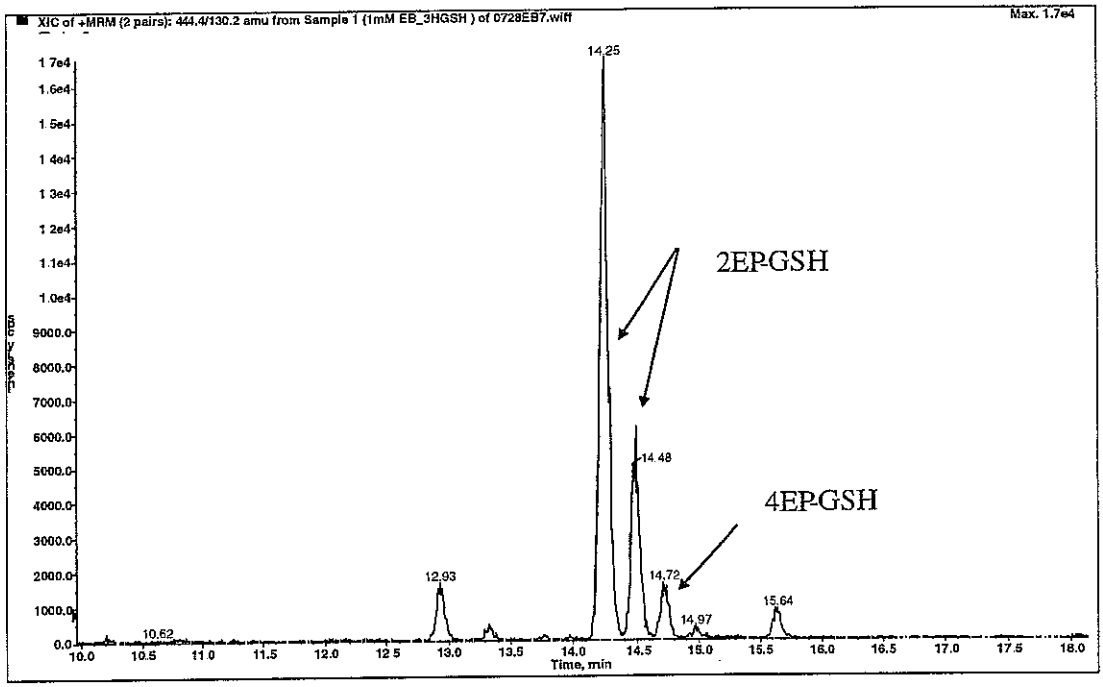
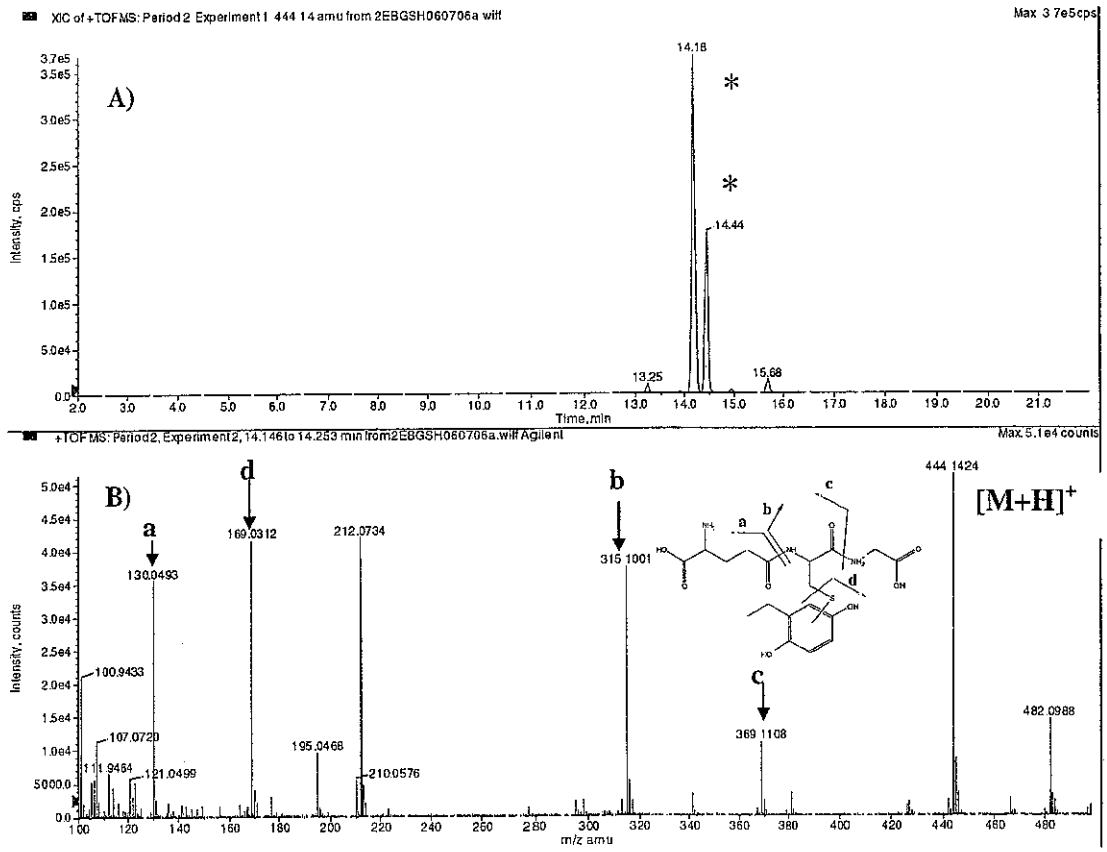


Figure 6 Representative HPLC/ESI-TOF-MS/MS Analysis of 2EP-GSH Conjugates

A) Total ion chromatogram of two positive-charged GSH conjugate isomers of 2EP-GSH; B) Product ion spectra of the GSH conjugate eluting at a retention time of 14 18 minutes



ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Figure 7. Representative HPLC/ESI-TOF-MS/MS Analysis of 4EP-GSH Conjugates

A) Total ion chromatogram of positive-charged GSH conjugate of 4EP; B) Product ion spectra of the GSH conjugate eluting at a retention time of 14.76 minutes

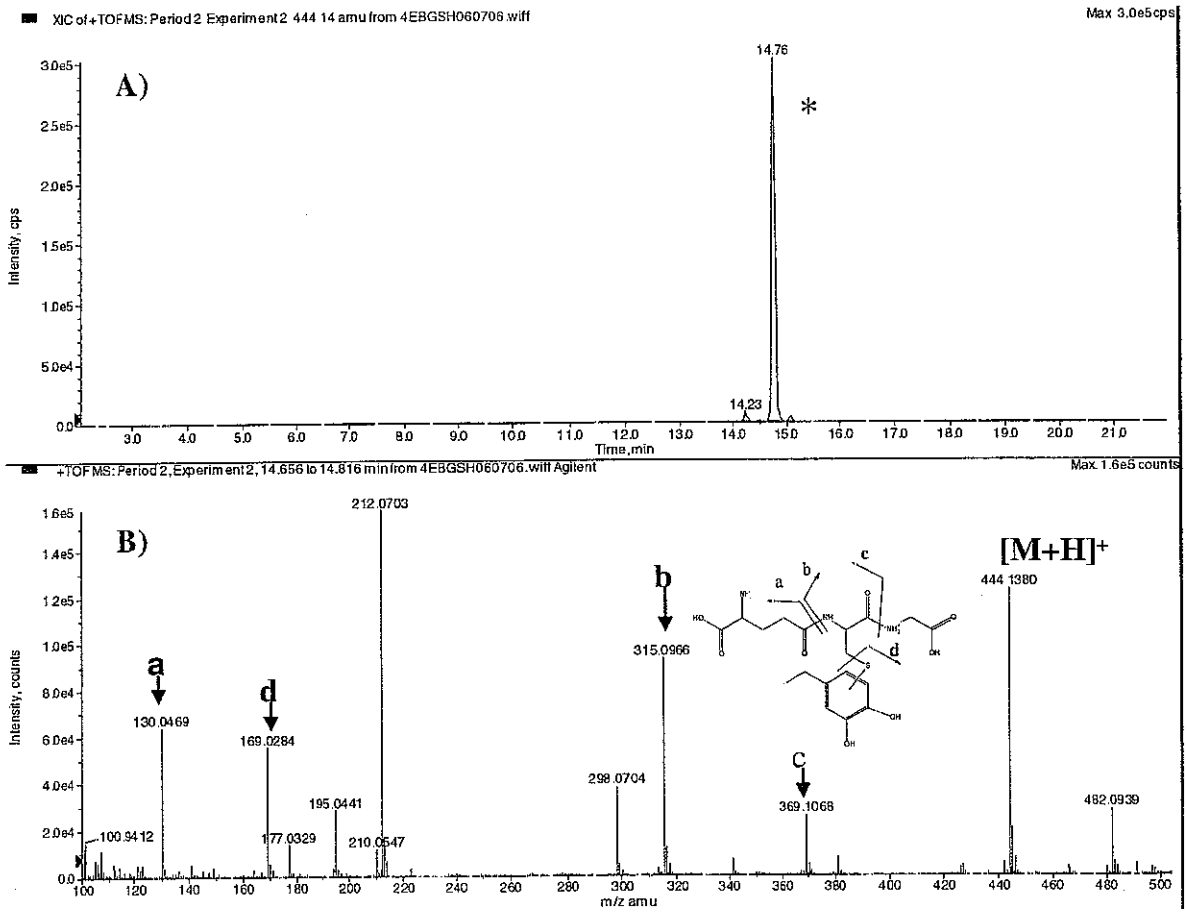


Figure 8. Graphs Showing Rates of Formation of 1-Phenylethanol and Acetophenone from Incubation of EB with Lung Microsomes (top), and Lineweaver-Burk Plot of Data used to Derive $K_m$ and $V_{max}$ values (bottom)

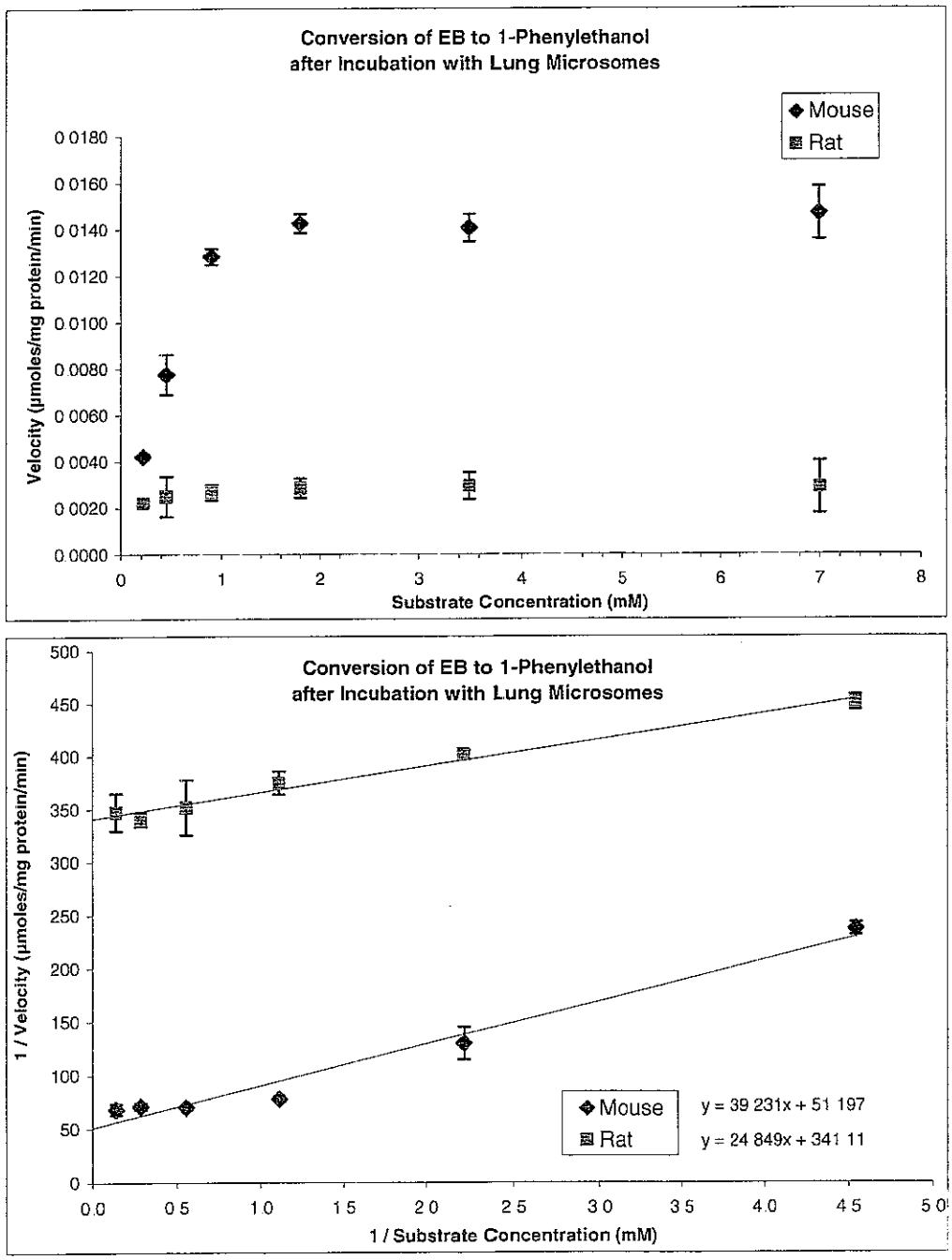
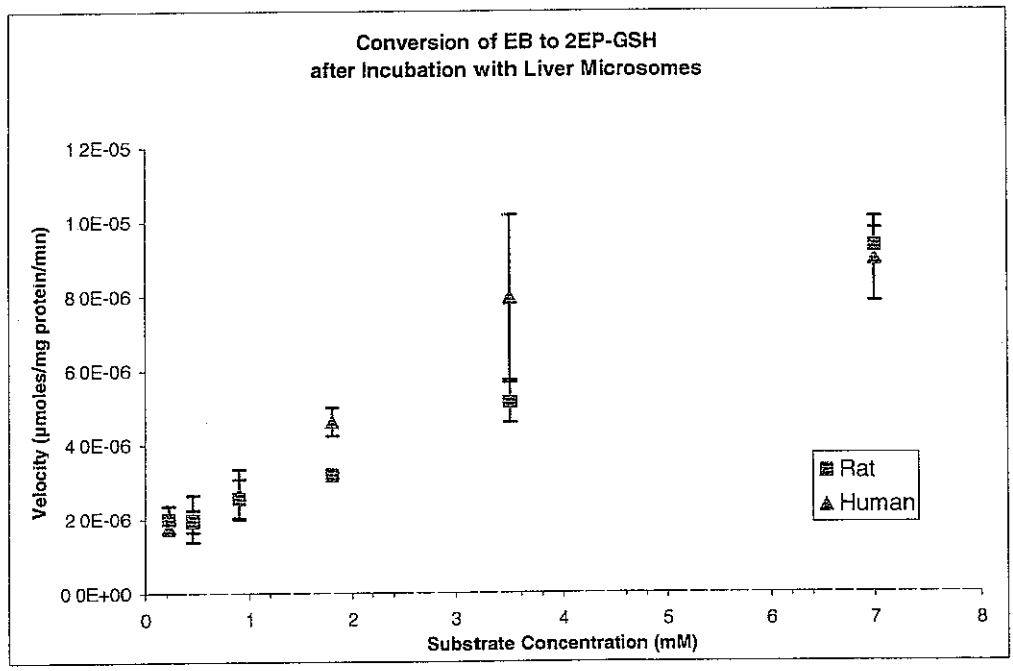
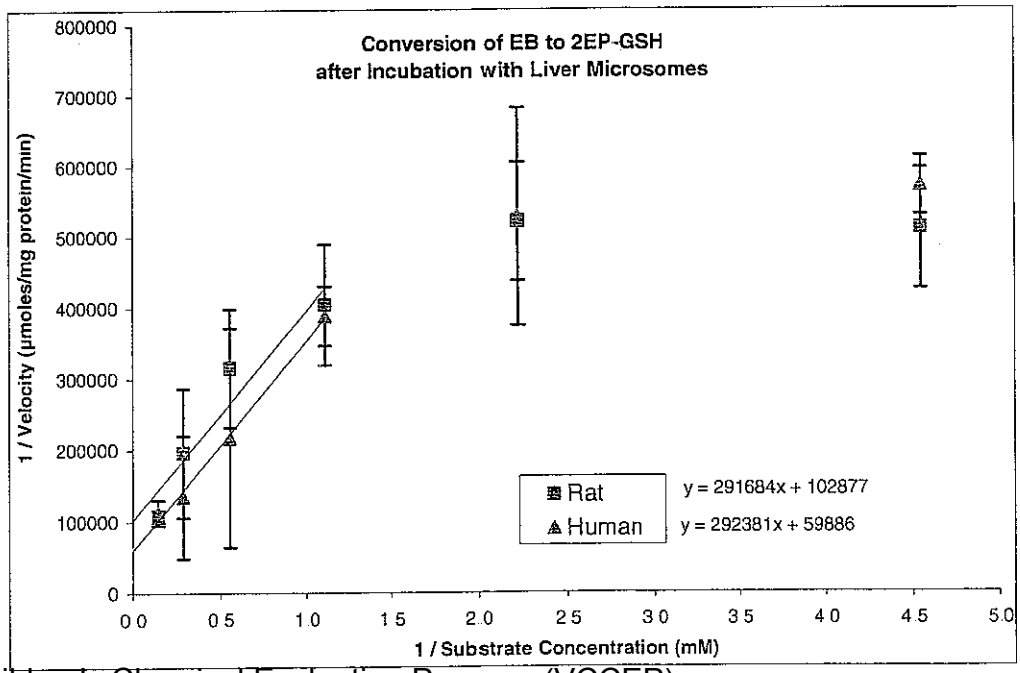


Figure 9 Graphs Showing Rates of Formation of 2EP-GSH from Incubation of EB with Liver Microsomes (top), and Lineweaver-Burk Plot of Data used to Derive $K_m$ and $V_{max}$ values (bottom)

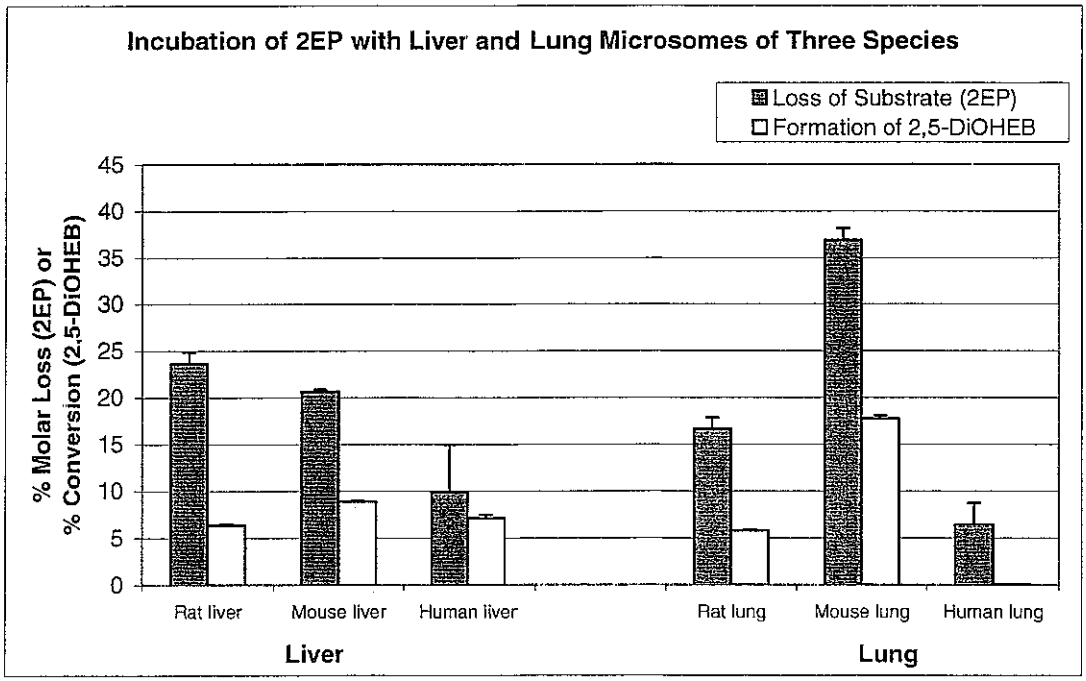


ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Table 1. Conversion of 2-Ethylphenol to Ethylhydroquinone after Incubation with Liver and Lung Microsomes from Mouse, Rat, and Human Tissues

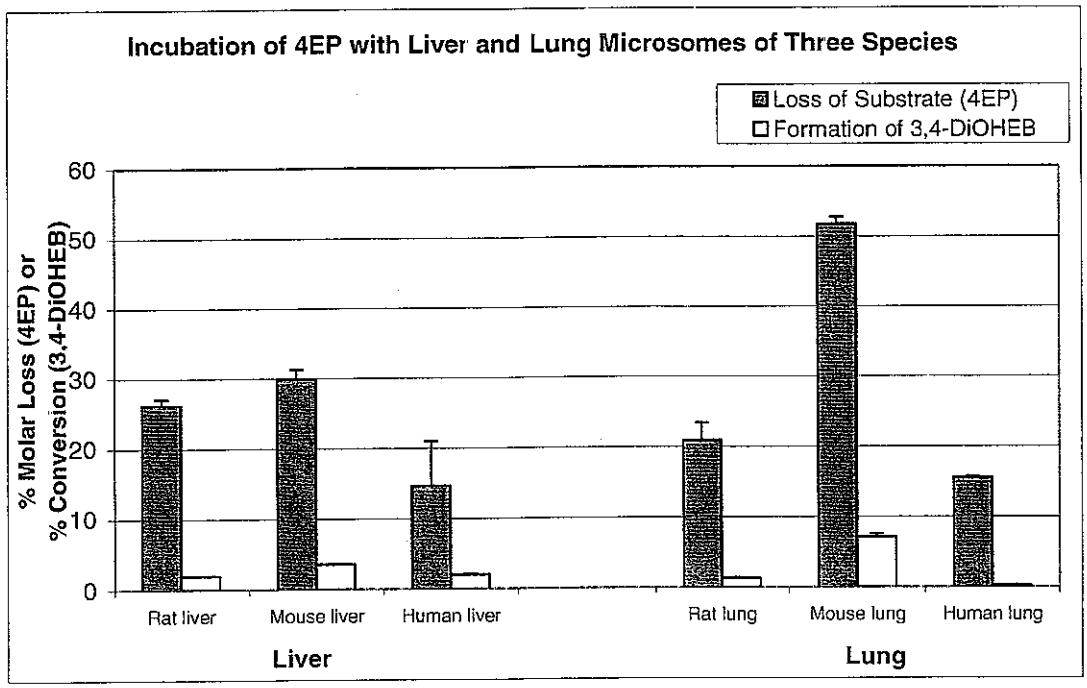
| | | 2-EP (substrate) | | Ethylhydroquinone | |
|---|---|---|---|---|---|
| Treatment | Amt 2-EP Loaded (µmoles) | Measured (µmoles) | Converted (%) | Measured (µmoles) | Conversion (%) |
| 1 mM; Mouse Liver | 0.98 | 0.778 | 20.6% | 0.0872 | 8.9% |
| 1 mM; Rat Liver | 0.98 | 0.749 | 23.6% | 0.0630 | 6.4% |
| 1 mM; Human Liver | 0.98 | 0.883 | 9.9% | 0.0700 | 7.1% |
| 1 mM; Mouse Lung | 0.98 | 0.619 | 36.8% | 0.173 | 17.7% |
| 1 mM; Rat Lung | 0.98 | 0.817 | 16.6% | 0.0569 | 5.8% |
| 1 mM; Human Lung | 0.98 | 0.917 | 6.4% | 0.0006 | 0.1% |



ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Table 2. Conversion of 4-Ethylphenol to Ethylcatechol after Incubation with Liver and Lung Microsomes from Mouse, Rat, and Human Tissues

| | | 4-EP (substrate) | | Ethylcatechol | |
|---|---|---|---|---|---|
| Treatment | Amt 4-EP Loaded (µmoles) | Measured (µmoles) | Converted (%) | Measured (µmoles) | Conversion (%) |
| 1 mM; Mouse Liver | 1.01 | 0.708 | 29.9% | 0.0359 | 3.6% |
| 1 mM; Rat Liver | 1.01 | 0.746 | 26.1% | 0.0200 | 2.0% |
| 1 mM; Human Liver | 1.01 | 0.862 | 14.7% | 0.0209 | 2.1% |
| 1 mM; Mouse Lung | 1.01 | 0.488 | 51.7% | 0.0721 | 7.1% |
| 1 mM; Rat Lung | 1.01 | 0.799 | 20.9% | 0.0137 | 1.4% |
| 1 mM; Human Lung | 1.01 | 0.853 | 15.5% | 0.0033 | 0.3% |

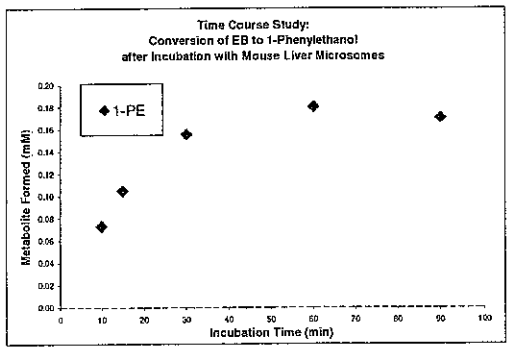


ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Table 3. Results of Time-Course Experiments to Assess Linearity of Metabolite Formation (conversion of EB to 1-Phenylethanol and Acetophenone)

Summary of Metabolites (1-Phenylethanol and Acetophenone) of EB Formed following Time-Course Experiment

| | 1-Phenylethanol | | | | Acetophenone | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Name | Conc (µg/mL) | Conc (mM) | Mean (mM) | Conversion (%) | Conc (µg/mL) | Conc (mM) | Mean (mM) | Conversion (%) |
| TC10a | 8.24 | 6.754E-02 | -- | -- | 0.206 | 1.717E-03 | -- | -- |
| TC10b | 9.57 | 7.844E-02 | 7.299E-02 | 3.6% | 0.279 | 2.325E-03 | 2.021E-03 | 0.10% |
| TC15a | 13.7 | 1.123E-01 | -- | -- | 0.357 | 2.975E-03 | -- | -- |
| TC15b | 11.9 | 9.754E-02 | 1.049E-01 | 5.2% | 0.360 | 3.000E-03 | 2.988E-03 | 0.15% |
| TC30a | 19.1 | 1.566E-01 | -- | -- | 0.734 | 6.117E-03 | -- | -- |
| TC30b | 18.9 | 1.549E-01 | 1.557E-01 | 7.8% | 0.730 | 6.083E-03 | 6.100E-03 | 0.31% |
| TC60a | 22.0 | 1.803E-01 | -- | -- | 1.44 | 1.200E-02 | -- | -- |
| TC60b | 22.0 | 1.803E-01 | 1.803E-01 | 9.0% | 1.21 | 1.008E-02 | 1.104E-02 | 0.55% |
| TC90a | 20.2 | 1.656E-01 | -- | -- | 1.52 | 1.267E-02 | -- | -- |
| TC90b | 21.4 | 1.754E-01 | 1.705E-01 | 8.5% | 1.44 | 1.200E-02 | 1.233E-02 | 0.62% |



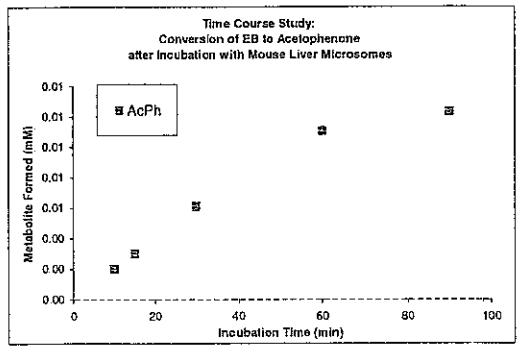



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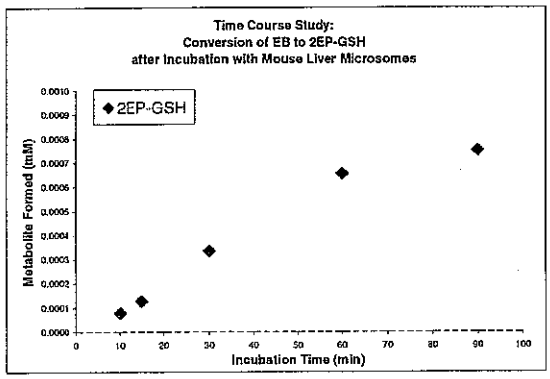
Table 4. Results of Time-Course Experiments to Assess Linearity of Metabolite Formation (conversion of EB to 2,5- and 3,4-ethylquinone via GSH trap)

| | 2EP-GSH | | | | 4EP-GSH | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Name | Conc (ng/mL) | Conc (mM) | Mean (mM) | Conversion (%) | Conc (ng/mL) | Conc (mM) | Mean (mM) | Conversion (%) |
| TC10a | 35 5 | 8.009E-05 | -- | -- | 2 38 | 5 363E-06 | -- | -- |
| TC10b | 34 1 | 7.695E-05 | 7 852E-05 | 0 004% | 1.65 | 3.733E-06 | 4.548E-06 | 0 00023% |
| TC15a | 56 9 | 1 284E-04 | -- | -- | 2 48 | 5 608E-06 | -- | -- |
| TC15b | 56 2 | 1 269E-04 | 1 276E-04 | 0 006% | 2 92 | 6 603E-06 | 6.105E-06 | 0 00031% |
| TC30a | 140 | 3 150E-04 | -- | -- | 6.21 | 1 403E-05 | -- | -- |
| TC30b | 157 | 3 554E-04 | 3 352E-04 | 0 017% | 6.78 | 1 531E-05 | 1 467E-05 | 0 00073% |
| TC60a | 327 | 7 383E-04 | -- | -- | 16.2 | 3 653E-05 | -- | -- |
| TC60b | 254 | 5 731E-04 | 6 557E-04 | 0 033% | 12 7 | 2.871E-05 | 3.262E-05 | 0 00163% |
| TC90a | 353 | 7 964E-04 | -- | -- | 18 5 | 4 177E-05 | -- | -- |
| TC90b | 313 | 7 071E-04 | 7 518E-04 | 0 038% | 14 7 | 3 325E-05 | 3 751E-05 | 0.00188% |



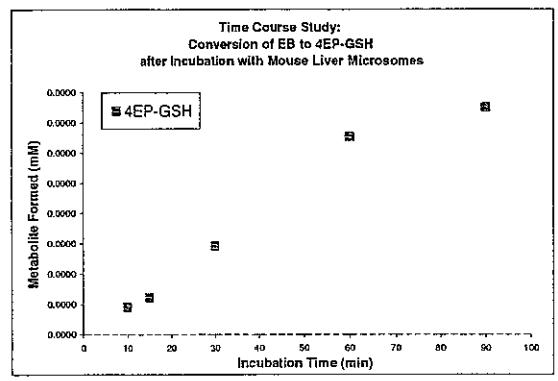



Table 5. Percent Conversion of Metabolites Formed in Incubations of Ethylbenzene with Liver Microsomes

| | | 1-Phenylethanol | | Acetophenone | | 2EP-GSH | | 4EP-GSH | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Amt EB Loaded (µmoles) | Measured (µmoles) | Conversion (%) | Measured (µmoles) | Conversion (%) | Measured (µmoles) | Conversion (%) | Measured (µmoles) | Conversion (%) |
| 0.22 mM; Mouse Liver | 0.22 | 7.45E-02 | 33.9% | 1.23E-02 | 5.57% | 6.34E-04 | 0.288% | 3.74E-05 | 0.0170% |
| 0.45 mM; Mouse Liver | 0.45 | 1.10E-01 | 24.4% | 1.13E-02 | 2.51% | 5.57E-04 | 0.124% | 3.12E-05 | 0.00693% |
| 0.90 mM; Mouse Liver | 0.90 | 1.44E-01 | 16.0% | 1.04E-02 | 1.16% | 4.61E-04 | 0.0512% | 2.25E-05 | 0.00250% |
| 1.8 mM; Mouse Liver | 1.8 | 1.46E-01 | 8.12% | 7.84E-03 | 0.436% | 3.67E-04 | 0.0204% | 1.33E-05 | 0.000741% |
| 3.5 mM; Mouse Liver | 3.5 | 1.49E-01 | 4.26% | 7.69E-03 | 0.220% | 3.49E-04 | 0.00997% | 1.07E-05 | 0.000306% |
| 7.0 mM; Mouse Liver | 7.0 | 1.74E-01 | 2.48% | 8.38E-03 | 0.120% | 4.33E-04 | 0.00618% | 7.35E-06 | 0.000105% |
| 0.22 mM; Rat Liver | 0.22 | 3.28E-02 | 14.9% | 4.10E-03 | 1.86% | 6.00E-05 | 0.0273% | 8.91E-06 | 0.00405% |
| 0.45 mM; Rat Liver | 0.45 | 4.60E-02 | 10.2% | 4.53E-03 | 1.01% | 5.85E-05 | 0.0130% | 3.73E-06 | 0.000829% |
| 0.90 mM; Rat Liver | 0.90 | 7.87E-02 | 8.75% | 8.30E-03 | 0.922% | 7.65E-05 | 0.00850% | 4.00E-06 | 0.000445% |
| 1.8 mM; Rat Liver | 1.8 | 1.24E-01 | 6.88% | 1.08E-02 | 0.599% | 9.53E-05 | 0.00529% | 3.78E-06 | 0.000210% |
| 3.5 mM; Rat Liver | 3.5 | 1.31E-01 | 3.73% | 9.79E-03 | 0.280% | 1.54E-04 | 0.00440% | 5.53E-06 | 0.000158% |
| 7.0 mM; Rat Liver | 7.0 | 1.46E-01 | 2.08% | 1.28E-02 | 0.182% | 2.80E-04 | 0.00399% | 1.10E-05 | 0.000157% |
| 0.22 mM; Human Liver | 0.22 | 3.77E-02 | 17.1% | <LLQ [b] | <1.36% | 5.27E-05 | 0.0240% | 8.74E-05 | 0.0397% |
| 0.45 mM; Human Liver | 0.45 | 4.80E-02 | 10.7% | 3.04E-03 | 0.676% | 6.04E-05 | 0.0134% | 7.38E-05 | 0.0164% |
| 0.90 mM; Human Liver | 0.90 | 5.88E-02 | 6.53% | 4.43E-03 | 0.492% | 8.03E-05 | 0.00892% | 5.86E-05 | 0.00651% |
| 1.8 mM; Human Liver | 1.8 | 6.62E-02 | 3.68% | <LLQ [b] | <0.167% | 1.39E-04 | 0.00770% | 4.25E-05 | 0.00236% |
| 3.5 mM; Human Liver | 3.5 | 7.49E-02 | 2.14% | 4.03E-03 | 0.115% | 2.39E-04 | 0.00682% | 2.03E-05 | 0.000581% |
| 7.0 mM; Human Liver | 7.0 | 8.91E-02 | 1.27% | 3.84E-03 | 0.0549% | 2.69E-04 | 0.00385% | 2.66E-05 | 0.000379% |

[a] <LLQ = Less than Lowest Level Quantified; LLQ = 0.360 µg (or 0.003 µmoles) 1-phenylethanol per incubation vial

[b] <LLQ = Less than Lowest Level Quantified; LLQ = 0.367 µg (or 0.003 µmoles) acetophenone per incubation vial

[c] <LLQ = Less than Lowest Level Quantified; LLQ = 2.32 ng (or $5 \times 10^{-6}$ µmole) 2EP-GSH per incubation vial

[d] <LLQ = Less than Lowest Level Quantified; LLQ = 1.64 ng (or $4 \times 10^{-6}$ µmole) 4EP-GSH per incubation vial

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Table 6. Percent Conversion of Metabolites Formed in Incubations of Ethylbenzene with Lung Microsomes

| | | 1-Phenylethanol | | Acetophenone | | 2EP-GSH | | 4EP-GSH | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Amt EB Loaded (µmoles)** | **Measured (µmoles)** | **Conversion (%)** | **Measured (µmoles)** | **Conversion (%)** | **Measured (µmoles)** | **Conversion (%)** | **Measured (µmoles)** | **Conversion (%)** |
| 0.22 mM; Mouse Lung | 0.22 | 1.27E-01 | 57.5% | 1.54E-02 | 7.0% | 1.21E-03 | 0.551% | 3.93E-05 | 0.0179% |
| 0.45 mM; Mouse Lung | 0.45 | 2.33E-01 | 51.7% | 2.17E-02 | 4.8% | 1.07E-03 | 0.237% | 5.79E-05 | 0.0129% |
| 0.90 mM; Mouse Lung | 0.90 | 3.85E-01 | 42.8% | 2.26E-02 | 2.5% | 4.94E-04 | 0.0549% | 4.17E-05 | 0.00463% |
| 1.8 mM; Mouse Lung | 1.8 | 4.28E-01 | 23.8% | 1.99E-02 | 1.1% | 2.93E-04 | 0.0163% | 2.50E-05 | 0.00139% |
| 3.5 mM; Mouse Lung | 3.5 | 4.22E-01 | 12.1% | 2.04E-02 | 0.6% | 2.43E-04 | 0.00695% | 2.07E-05 | 0.000593% |
| 7.0 mM; Mouse Lung | 7.0 | 4.41E-01 | 6.29% | 2.02E-02 | 0.3% | 1.88E-04 | 0.00268% | 1.40E-05 | 0.000200% |
| 0.22 mM; Rat Lung | 0.22 | 6.66E-02 | 30.3% | 3.05E-03 | 1.4% | 1.29E-04 | 0.0587% | 2.65E-05 | 0.0120% |
| 0.45 mM; Rat Lung | 0.45 | 7.47E-02 | 16.6% | <LLQ [b] | <0.7% | 1.01E-04 | 0.0225% | 1.95E-05 | 0.00434% |
| 0.90 mM; Rat Lung | 0.90 | 8.01E-02 | 8.90% | <LLQ [b] | <0.3% | 7.65E-05 | 0.00850% | 1.47E-05 | 0.00164% |
| 1.8 mM; Rat Lung | 1.8 | 8.57E-02 | 4.76% | <LLQ [b] | <0.2% | 5.82E-05 | 0.00323% | 1.14E-05 | 0.000632% |
| 3.5 mM; Rat Lung | 3.5 | 8.81E-02 | 2.52% | <LLQ [b] | <0.1% | 5.09E-05 | 0.00145% | 8.95E-06 | 0.000256% |
| 7.0 mM; Rat Lung | 7.0 | 8.65E-02 | 1.24% | <LLQ [b] | <0.04% | 4.09E-05 | 0.000585% | 6.65E-06 | 0.0000950% |
| 0.22 mM; Human Lung | 0.22 | <LLQ [a] | <1.4% | <LLQ [b] | <1.4% | <LLQ [c] | <0.002% | <LLQ [d] | <0.002% |
| 0.45 mM; Human Lung | 0.45 | <LLQ [a] | <0.7% | <LLQ [b] | <0.7% | <LLQ [c] | <0.001% | <LLQ [d] | <0.001% |
| 0.90 mM; Human Lung | 0.90 | <LLQ [a] | <0.3% | <LLQ [b] | <0.3% | <LLQ [c] | <0.0006% | <LLQ [d] | <0.0004% |
| 1.8 mM; Human Lung | 1.8 | <LLQ [a] | <0.2% | <LLQ [b] | <0.2% | <LLQ [c] | <0.0003% | <LLQ [d] | <0.0002% |
| 3.5 mM; Human Lung | 3.5 | <LLQ [a] | <0.1% | <LLQ [b] | <0.1% | <LLQ [c] | <0.0001% | <LLQ [d] | <0.0001% |
| 7.0 mM; Human Lung | 7.0 | <LLQ [a] | <0.04% | <LLQ [b] | <0.0% | <LLQ [c] | <0.00007% | <LLQ [d] | <0.0001% |

[a] <LLQ = Less than Lowest Level Quantified; LLQ = 0.360 µg (or 0.003 µmoles) 1-phenylethanol per incubation vial
[b] <LLQ = Less than Lowest Level Quantified; LLQ = 0.367 µg (or 0.003 µmoles) acetophenone per incubation vial
[c] <LLQ = Less than Lowest Level Quantified; LLQ = 2.32 ng (or 5 x $10^{-6}$ µmole) 2EP-GSH per incubation vial
[d] <LLQ = Less than Lowest Level Quantified; LLQ = 1.64 ng (or 4 x $10^{-6}$ µmole) 4EP-GSH per incubation vial

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Table 7. $K_m$ and $V_{max}$ Values Derived for Metabolites whose Rates of Conversion Increase with Increasing Substrate Concentration: Values for Formation of 1-Phenylethanol and Acetophenone (Top), and 2EP-GSH and 4EP-GSH (Bottom)

| | 1-Phenylethanol | | Acetophenone | | 1-PE + AcPh composite | |
|---|---|---|---|---|---|---|
| Treatment | Km (mM) | Vmax (µmoles/mg/min) | Km (mM) | Vmax (µmoles/mg/min) | Km (mM) | Vmax (µmoles/mg/min) |
| Mouse Liver | 0.288 | 0.00578 | NA_Decr [a] | NA_Decr [a] | 0.230 | 0.00595 |
| Rat Liver | 0.952 | 0.00538 | 0.498 | 0.000395 | 0.897 | 0.00575 |
| Human Liver | 0.273 | 0.00265 | 0.150 | 0.000148 | 0.290 | 0.00276 |
| Mouse Lung | 0.766 | 0.0195 | 0.0694 | 0.000722 | 0.670 | 0.0198 |
| Rat Lung | 0.0727 | 0.00293 | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] |
| Human Lung | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] |

[a] NA_Decr: Not Applicable, as amount of metabolite produced decreased with increasing substrate (EB) concentration

[b] NA_<LLQ: Not Applicable, as metabolite was not detected in incubation solutions

| | 2EP-GSH | | 4EP-GSH | | 2EP-GSH + 4EP-GSH composite | |
|---|---|---|---|---|---|---|
| Treatment | Km (mM) | Vmax (µmoles/mg/min) | Km (mM) | Vmax (µmoles/mg/min) | Km (mM) | Vmax (µmoles/mg/min) |
| Mouse Liver | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] |
| Rat Liver | 2.84 | 9.72E-06 | 1.86 | 3.78E-07 | 1.86 | 3.78E-07 |
| Human Liver | 4.88 | 1.67E-05 | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] | NA_Decr [a] |

[a] NA_Decr: Not Applicable, as amount of metabolite produced decreased with increasing substrate (EB) concentration

Note: Km and Vmax not calculated for rates of conversion of EB to 2EP-GSH and 4EP-GSH in lung microsomes incubations because these metabolites decreased with increasing substrate concentrations in incubations with mouse and rat lung microsomes; and were not detected following incubations with human lung microsomes

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Table 8. Maximum and Minimum Rates of Formation (µmole/mg protein/minute) for Metabolites whose Rates of Conversion Decrease with Increasing Substrate Concentration (unable to construct convential Lineweaver-Burk plots)

| | 2EP-GSH | | 4EP-GSH | |
|---|---|---|---|---|
| Treatment | Max Velocity (µmoles/mg/min) | Min Velocity (µmoles/mg/min) | Max Velocity (µmoles/mg/min) | Min Velocity (µmoles/mg/min) |
| Mouse Liver | 2.11E-05 | 1.44E-05 | 1.25E-06 | 2.45E-07 |
| Human Liver | NA_Incr [a] | NA_Incr [a] | 2.91E-06 | 8.85E-07 |
| Mouse Lung | 4.04E-05 | 6.25E-06 | 1.93E-06 | 4.67E-07 |
| Rat Lung | 4.30E-06 | 1.36E-06 | 8.82E-07 | 2.22E-07 |
| Human Lung | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] | NA_<LLQ [b] |

[a] NA_Incr: Not Applicable as amount of metabolite produced increased with increasing substrate (EB) concentration and Km Vmax values were derived

[b] NA_<LLQ: Not Applicable as metabolite was not detected in incubation solutions

**Note: For these metabolites (2EP-GSH and 4EP-GSH) produced in these microsome systems, amounts of metabolites decreased with increasing substrate concentrations. Except for formation of 4EP-GSH from mouse lung incubations, the maximum velocity occurred at the lowest (0.22 mM) EB concentration, and the highest velocity always occurred for the highest (7 mM) substrate concentration. The Max Velocity for 4EP-GSH formation in mouse lung incubations occurred at the 0.45 mM substrate level.**

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Appendix Table 1. Individual Values from GC/MS Analysis of Liver Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone

| Treatment | Mouse Liver Incubations | | | Rat Liver Incubations | | | Human Liver Incubations | | |
|---|---|---|---|---|---|---|---|---|---|
| | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) |
| Control no microsomes | 57 2 [a] | <LLQ [c] | <LLQ [d] | | | | | | |
| Control no substrate | <LLQ [b] | <LLQ [c] | <LLQ [d] | <LLQ [b] | <LLQ [c] | <LLQ [d] | <LLQ [b] | <LLQ [c] | 0 452 |
| 0 45 no NADPH | 44 8 | <LLQ [c] | <LLQ [d] | 46 4 | <LLQ [c] | <LLQ [d] | 44 9 | <LLQ [c] | <LLQ [d] |
| 1 8 no NADPH | 182 | <LLQ [c] | <LLQ [d] | 202 | <LLQ [c] | <LLQ [d] | 202 | <LLQ [c] | <LLQ [d] |
| 7 no NADPH | 737 | <LLQ [c] | <LLQ [d] | 789 | <LLQ [c] | <LLQ [d] | 783 | <LLQ [c] | <LLQ [d] |
| 0 22a | 5 93 | 10 2 | 1 42 | 32 1 | 5 33 | 0 664 | 19 6 | 5 93 | <LLQ [d] |
| 0 22b | 10 3 | 8 88 | 1 57 | 24 8 | 3 46 | 0 406 | 18 2 | 3 71 | <LLQ [d] |
| 0 22c | 16 4 | 8 23 | 1 43 | 22 7 | 3 24 | 0 407 | 20 6 | 4 19 | <LLQ [d] |
| Average: | **10.9** | **9.10** | **1.47** | **26.5** | **4.01** | **0.492** | **19.5** | **4.61** | <LLQ [d] |
| %RSD: | 48.3% | 11.0% | 5.69% | 18.6% | 28.6% | 30.20% | 6.19% | 25.3% | |
| 0 45a | 40 7 | 14 0 | 1 44 | 32 2 | 6 00 | 0 533 | 29 3 | 6 14 | <LLQ [d] |
| 0 45b | 34 7 | 13 9 | 1 38 | 36 9 | 5 71 | 0 592 | 37 0 | 6 13 | <LLQ [d] |
| 0 45c | 32 3 | 12 4 | 1 25 | 33 6 | 5 16 | 0 509 | 29 3 | 5 33 | 0 376 |
| Average: | **35.9** | **13.4** | **1.36** | **34.2** | **5.62** | **0.545** | **31.9** | **5.87** | **0.370** |
| %RSD: | 12.1% | 6.67% | 7.16% | 7.05% | 7.59% | 7.84% | 14.0% | 7.92% | 1.4% |
| 0 9a | 111 | 22 4 | 1 56 | No Peak [e] | 9 30 | 0 979 | 102 | 7 87 | 0 391 |
| 0 9b | 74 8 | 15 9 | 1 18 | 91 5 | 10 0 | 0 904 | 66 6 | 6 13 | <LLQ [d] |
| 0 9c | 83 8 | 14 4 | 1 01 | 96 3 | 9 56 | 1 11 | 92 0 | 7 55 | 0 845 |
| Average: | **89.9** | **17.6** | **1.25** | **93.9** | **9.62** | **1.00** | **86.9** | **7.18** | **0.534** |
| %RSD: | 21.0% | 24.2% | 22.5% | NA (n=2) | 3.68% | 10.5% | 21.0% | 12.9% | 50.4% |
| 1 8a | 170 | 17 8 | 0 934 | 264 | 19 3 | 1 71 | 142 | 7 97 | <LLQ [d] |
| 1 8b | 175 | 18 1 | 0 907 | 185 | 12 9 | 1 08 | 145 | 8 03 | <LLQ [d] |
| 1 8c | 187 | 17 7 | 0 987 | 199 | 13 2 | 1 10 | 130 | 8 26 | <LLQ [d] |
| Average: | **177** | **17.9** | **0.943** | **216** | **15.1** | **1 30** | **139** | **8.09** | <LLQ [d] |
| %RSD: | 4.93% | 1.17% | 4.32% | 19.5% | 23.9% | 27.6% | 5.71% | 1.89% | |
| 3 5a | 391 | 19.1 | 1.01 | 286 | 17.5 | 1 19 | 313 | 8.69 | 0.490 |
| 3 5b | 386 | 17 8 | 0.858 | 269 | 16 1 | 1 25 | 293 | 10.5 | 0.601 |
| 3 5c | 306 | 17.8 | 0.904 | 268 | 14.3 | 1.09 | 246 | 8.28 | <LLQ [d] |
| Average: | **361** | **18.2** | **0.924** | **274** | **16.0** | **1.18** | **284** | **9.16** | **0.486** |
| %RSD: | 13.2% | 4.12% | 8.44% | 3.69% | 10.0% | 6.87% | 12.1% | 12.9% | 24.1% |
| 7a | 745 | 25.8 | 1.20 | NS | 19.7 | 1 59 | 611 | 12.4 | 0.401 |
| 7b | 698 | 19 4 | 0 916 | 647 | 17 6 | 1 57 | NS | 11.8 | 0 473 |
| 7c | 664 | 18.5 | 0.906 | 598 | 16.1 | 1.44 | 538 | 8.48 | 0.511 |
| Average: | **702** | **21.2** | **1.01** | **623** | **17.8** | **1.53** | **575** | **10.9** | **0.462** |
| %RSD: | 5.79% | 18.7% | 16.57% | NA | 10.2% | 5.31% | NA | 19.4% | 12.1% |

[a] Data collected in initial analysis for ethylbenzene where systemic ethylbenzene contamination was observed

[b] <LLQ = less than lowest level quantitated = 2 32 µg ethylbenzene/mL

[c] <LLQ = less than lowest level quantitated = 0 360 µg 1-phenylethanol/ml

[d] <LLQ = less than lowest level quantitated = 0 367 µg acetophenone/mL

[e] No peaks for either ethylbenzene or internal standard - suspect injection error

NS = No Sample

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 1. Individual Values from GC/MS Analysis of Liver Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Mouse Liver Incubations (from 9/21/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) (µmole / vessel) | EB (µg/mL) | EB (µmole) | EB (%Conv) | 1-PE (µg/mL) | 1-PE (µmole) | 1-PE (%Conv) | AcPh (µg/mL) | AcPh (µmole) | AcPh (%Conv) | EB+1-PE+AcPh (mass balance) (µmole) | EB+1-PE+AcPh (mass balance) (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.22a | 5.93 | 0.0558 | 25.4% | 10.2 | 0.0835 | 37.9% | 1.42 | 0.0118 | 5.4% | 0.1511 | 68.7% |
| 0.22b | 10.3 | 0.0970 | 44.1% | 8.88 | 0.0727 | 33.0% | 1.57 | 0.0131 | 5.9% | 0.1827 | 83.1% |
| 0.22c | 16.4 | 0.1544 | 70.2% | 8.23 | 0.0673 | 30.6% | 1.43 | 0.0119 | 5.4% | 0.2337 | 106.2% |
| **Mean** | **10.9** | **1.02E-01** | **46.6%** | **9.10** | **7.45E-02** | **33.9%** | **1.47** | **1.23E-02** | **5.6%** | **0.189** | **86.0%** |
| **Std. Dev** | **5.26** | **4.95E-02** | **22.5%** | **1.00** | **8.21E-03** | **3.7%** | **0.0839** | **6.98E-04** | **0.3%** | **0.0417** | **18.9%** |
| 0.45a | 40.7 | 0.3832 | 85.2% | 14.0 | 0.1146 | 25.5% | 1.44 | 0.0120 | 2.7% | 0.5098 | 113.3% |
| 0.45b | 34.7 | 0.3267 | 72.6% | 13.9 | 0.1137 | 25.3% | 1.38 | 0.0115 | 2.6% | 0.4520 | 100.4% |
| 0.45c | 32.3 | 0.3041 | 67.6% | 12.4 | 0.1015 | 22.5% | 1.25 | 0.0104 | 2.3% | 0.4160 | 92.4% |
| **Mean** | **35.9** | **3.38E-01** | **75.1%** | **13.4** | **1.10E-01** | **24.4%** | **1.36** | **1.13E-02** | **2.5%** | **0.459** | **102.1%** |
| **Std. Dev** | **4.33** | **4.07E-02** | **9.1%** | **0.896** | **7.33E-03** | **1.6%** | **0.0971** | **8.08E-04** | **0.2%** | **0.0473** | **10.5%** |
| 0.9a | 111 | 1.0452 | 116% | 22.4 | 0.1833 | 20.4% | 1.56 | 0.0130 | 1.4% | 1.2415 | 137.9% |
| 0.9b | 74.8 | 0.7043 | 78.3% | 15.9 | 0.1301 | 14.5% | 1.18 | 0.0098 | 1.1% | 0.8443 | 93.8% |
| 0.9c | 83.8 | 0.7891 | 87.7% | 14.4 | 0.1178 | 13.1% | 1.01 | 0.0084 | 0.9% | 0.9153 | 101.7% |
| **Mean** | **89.9** | **8.46E-01** | **94.0%** | **17.6** | **1.44E-01** | **16.0%** | **1.25** | **1.04E-02** | **1.2%** | **1.00** | **111.2%** |
| **Std. Dev** | **18.8** | **1.77E-01** | **19.7%** | **4.25** | **3.48E-02** | **3.9%** | **0.282** | **2.34E-03** | **0.3%** | **0.212** | **23.5%** |
| 1.8a | 170 | 1.6008 | 88.9% | 17.8 | 0.1457 | 8.1% | 0.934 | 0.0078 | 0.4% | 1.7542 | 97.5% |
| 1.8b | 175 | 1.6478 | 91.5% | 18.1 | 0.1481 | 8.2% | 0.907 | 0.0075 | 0.4% | 1.8035 | 100.2% |
| 1.8c | 187 | 1.7608 | 97.8% | 17.7 | 0.1448 | 8.0% | 0.987 | 0.0082 | 0.5% | 1.9139 | 106.3% |
| **Mean** | **177** | **1.67E+00** | **92.8%** | **17.9** | **1.46E-01** | **8.1%** | **0.943** | **7.84E-03** | **0.4%** | **1.824** | **101.3%** |
| **Std. Dev** | **8.74** | **8.23E-02** | **4.6%** | **0.208** | **1.70E-03** | **0.1%** | **0.0407** | **3.39E-04** | **0.02%** | **0.0818** | **4.5%** |
| 3.5a | 391 | 3.6817 | 105% | 19.1 | 0.1563 | 4.5% | 1.01 | 0.0084 | 0.2% | 3.8464 | 109.9% |
| 3.5b | 386 | 3.6347 | 104% | 17.8 | 0.1457 | 4.2% | 0.858 | 0.0071 | 0.2% | 3.7875 | 108.2% |
| 3.5c | 306 | 2.8814 | 82.3% | 17.8 | 0.1457 | 4.2% | 0.904 | 0.0075 | 0.2% | 3.0345 | 86.7% |
| **Mean** | **361** | **3.40E+00** | **97.1%** | **18.2** | **1.49E-01** | **4.3%** | **0.924** | **7.69E-03** | **0.2%** | **3.556** | **101.6%** |
| **Std. Dev** | **47.7** | **4.49E-01** | **12.8%** | **0.751** | **6.14E-03** | **0.2%** | **0.0779** | **6.48E-04** | **0.02%** | **0.453** | **12.9%** |
| 7a | 745 | 7.0151 | 100% | 25.8 | 0.2111 | 3.0% | 1.20 | 0.0100 | 0.1% | 7.2362 | 103.4% |
| 7b | 698 | 6.5725 | 93.9% | 19.4 | 0.1588 | 2.3% | 0.916 | 0.0076 | 0.1% | 6.7389 | 96.3% |
| 7c | 664 | 6.2524 | 89.3% | 18.5 | 0.1514 | 2.2% | 0.906 | 0.0075 | 0.1% | 6.4113 | 91.6% |
| **Mean** | **702** | **6.61E+00** | **94.5%** | **21.2** | **1.74E-01** | **2.5%** | **1.01** | **8.38E-03** | **0.1%** | **6.795** | **97.1%** |
| **Std. Dev** | **40.7** | **3.83E-01** | **5.5%** | **3.98** | **3.26E-02** | **0.5%** | **0.167** | **1.39E-03** | **0.02%** | **0.415** | **5.9%** |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 1 Individual Values from GC/MS Analysis of Liver Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Rat Liver Incubations (from 9/21/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) | EB | | | 1-PE | | | AcPh | | | EB+1-PE+AcPh (mass balance) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (µmole / vessel) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µmole) | (%) |
| 0 22a | 32 1 | 0 3023 | 137% | 5.33 | 0 0436 | 19 8% | 0 664 | 0.0055 | 2 5% | 0 3514 | 159 7% |
| 0 22b | 24 8 | 0 2335 | 106% | 3.46 | 0 0283 | 12 9% | 0 406 | 0.0034 | 1 5% | 0 2652 | 120 6% |
| 0 22c | 22 7 | 0 2137 | 97 2% | 3.24 | 0 0265 | 12 1% | 0 407 | 0 0034 | 1 5% | 0 2436 | 110.7% |
| **Mean** | **26.5** | **2 50E-01** | **113.6%** | **4.01** | **3.28E-02** | **14.9%** | **0.492** | **4.10E-03** | **1 9%** | **0.287** | **130.3%** |
| **Std. Dev** | **4.93** | **4.65E-02** | **21.1%** | **1.15** | **9.40E-03** | **4.3%** | **0.149** | **1.24E-03** | **0.6%** | **0.0570** | **25.9%** |
| 0 45a | 32 2 | 0 3032 | 67.4% | 6.00 | 0.0491 | 10 9% | 0 533 | 0 0044 | 1.0% | 0 3567 | 79 3% |
| 0.45b | 36 9 | 0.3475 | 77.2% | 5.71 | 0.0467 | 10.4% | 0 592 | 0 0049 | 1 1% | 0 3991 | 88 7% |
| 0.45c | 33 6 | 0.3164 | 70 3% | 5.16 | 0.0422 | 9 4% | 0 509 | 0.0042 | 0 9% | 0.3628 | 80.6% |
| **Mean** | **34.2** | **3.22E-01** | **71.6%** | **5.62** | **4.60E-02** | **10.2%** | **0.545** | **4.53E-03** | **1.0%** | **0.373** | **82.9%** |
| **Std. Dev** | **2.41** | **2.27E-02** | **5.0%** | **0.427** | **3.49E-03** | **0.8%** | **0.0427** | **3.55E-04** | **0.1%** | **0.0229** | **5.1%** |
| 0 9a | No Peak | NA | NA | 9.30 | 0.0761 | 8.5% | 0 979 | 0.0081 | 0 9% | 0.0842 | NA |
| 0 9b | 91 5 | 0.8616 | 95 7% | 10.00 | 0.0818 | 9.1% | 0 904 | 0.0075 | 0.8% | 0 9509 | 105 7% |
| 0 9c | 96 3 | 0 9068 | 101% | 9 56 | 0.0782 | 8 7% | 1.11 | 0.0092 | 1.0% | 0 9942 | 110.5% |
| **Mean** | **93.9** | **8.84E-01** | **98.2%** | **9.62** | **7 87E-02** | **8.7%** | **1.00** | **8.30E-03** | **0 9%** | **0.676** | **108.1%** |
| **Std. Dev** | NA (n=2) | **3.20E-02** | **3.6%** | **0.354** | **2.90E-03** | **0.3%** | **0.104** | **8.67E-04** | **0.1%** | **0.513** | **3.4%** |
| 1 8a | 264 | 2 4859 | 138% | 19 3 | 0 1579 | 8 8% | 1.71 | 0.0142 | 0.8% | 2.6580 | 147.7% |
| 1.8b | 185 | 1 7420 | 96 8% | 12 9 | 0 1056 | 5 9% | 1.08 | 0.0090 | 0.5% | 1 8565 | 103.1% |
| 1 8c | 199 | 1.8738 | 104% | 13 2 | 0 1080 | 6.0% | 1 10 | 0.0092 | 0.5% | 1 9910 | 110.6% |
| **Mean** | **216** | **2.03E+00** | **113.0%** | **15.1** | **1 24E-01** | **6 9%** | **1.30** | **1.08E-02** | **0.6%** | **2.17** | **120.5%** |
| **Std. Dev** | **42.2** | **3.97E-01** | **22.1%** | **3.61** | **2.96E-02** | **1.6%** | **0.358** | **2.98E-03** | **0.2%** | **0.429** | **23.8%** |
| 3 5a | 286 | 2.6930 | 76 9% | 17 5 | 0 1432 | 4 1% | 1 19 | 0.0099 | 0 3% | 2.8461 | 81 3% |
| 3 5b | 269 | 2 5330 | 72.4% | 16 1 | 0 1318 | 3.8% | 1 25 | 0.0104 | 0 3% | 2.6751 | 76 4% |
| 3 5c | 268 | 2 5235 | 72 1% | 14 3 | 0 1170 | 3 3% | 1 09 | 0.0091 | 0 3% | 2.6496 | 75 7% |
| **Mean** | **274** | **2.58E+00** | **73.8%** | **16.0** | **1 31E-01** | **3.7%** | **1.18** | **9 79E-03** | **0.3%** | **2.72** | **77.8%** |
| **Std. Dev** | **10.1** | **9.53E-02** | **2.7%** | **1.60** | **1.31E-02** | **0.4%** | **0.0808** | **6.72E-04** | **0.02%** | **0.107** | **3.1%** |
| 7a | NS | NA | NA | 19 7 | 0.1612 | 2 3% | 1 59 | 0.0132 | 0 2% | 0 1744 | 2.5% |
| 7b | 647 | 6.0923 | 87 0% | 17.6 | 0 1440 | 2 1% | 1 57 | 0.0131 | 0 2% | 6 2494 | 89 3% |
| 7c | 598 | 5.6309 | 80 4% | 16 1 | 0 1318 | 1 9% | 1 44 | 0.0120 | 0 2% | 5 7746 | 82 5% |
| **Mean** | **623** | **5.86E+00** | **83.7%** | **17.8** | **1.46E-01** | **2.1%** | **1.53** | **1 28E-02** | **0.2%** | **4.07** | **58.1%** |
| **Std. Dev** | **NA** | **3.26E-01** | **NA** | **1.81** | **1.48E-02** | **0.2%** | **0.0814** | **6.78E-04** | **0.01%** | **3.379** | **48.3%** |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 1. Individual Values from GC/MS Analysis of Liver Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Human Liver Incubations (from 9/21/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) (µmole / vessel) | EB (µg/mL) | EB (µmole) | EB (%Conv) | 1-PE (µg/mL) | 1-PE (µmole) | 1-PE (%Conv) | AcPh (µg/mL) | AcPh (µmole) | AcPh (%Conv) | EB+1-PE+AcPh (mass balance) (µmole) | EB+1-PE+AcPh (mass balance) (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.22a | 19.6 | 0.1846 | 83.9% | 5.93 | 0.0485 | 22.1% | <LLQ [b] | <LLQ [b] | NA | 0.2331 | 105.9% |
| 0.22b | 18.2 | 0.1714 | 77.9% | 3.71 | 0.0304 | 13.8% | <LLQ [b] | <LLQ [b] | NA | 0.2017 | 91.7% |
| 0.22c | 20.6 | 0.1940 | 88.2% | 4.19 | 0.0343 | 15.6% | <LLQ [b] | <LLQ [b] | NA | 0.2283 | 103.8% |
| **Mean** | **19.5** | **1.83E-01** | **83.3%** | **4.61** | **3.77E-02** | **17.1%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.221** | **100.5%** |
| **Std. Dev** | **1.21** | **1.14E-02** | **5.2%** | **1.17** | **9.56E-03** | **4.3%** | | | **NA** | **0.0169** | **7.7%** |
| 0.45a | 29.3 | 0.2759 | 61.3% | 6.14 | 0.0502 | 11.2% | <LLQ [b] | <LLQ [b] | NA | 0.3261 | 72.5% |
| 0.45b | 37.0 | 0.3484 | 77.4% | 6.13 | 0.0502 | 11.1% | <LLQ [b] | <LLQ [b] | NA | 0.3986 | 88.6% |
| 0.45c | 29.3 | 0.2759 | 61.3% | 5.33 | 0.0436 | 9.7% | 0.376 | 0.0031 | 0.7% | 0.3226 | 71.7% |
| **Mean** | **31.9** | **3.00E-01** | **66.7%** | **5.87** | **4.80E-02** | **10.7%** | **0.370** | **3.04E-03** | **0.7%** | **0.349** | **77.6%** |
| **Std. Dev** | **4.45** | **4.19E-02** | **9.3%** | **0.465** | **3.80E-03** | **0.8%** | **0.00520** | **7.40E-05** | **0.02%** | **0.0429** | **9.5%** |
| 0.9a | 102 | 0.9605 | 107% | 7.87 | 0.0644 | 7.2% | 0.391 | 0.0033 | 0.4% | 1.0281 | 114.2% |
| 0.9b | 66.6 | 0.6271 | 69.7% | 6.13 | 0.0502 | 5.6% | <LLQ [b] | <LLQ [b] | NA | 0.6773 | 75.3% |
| 0.9c | 92.0 | 0.8663 | 96.3% | 7.55 | 0.0618 | 6.9% | 0.845 | 0.0070 | 0.8% | 0.9351 | 103.9% |
| **Mean** | **86.9** | **8.18E-01** | **90.9%** | **7.18** | **5.88E-02** | **6.5%** | **0.534** | **4.43E-03** | **0.5%** | **0.880** | **97.8%** |
| **Std. Dev** | **18.2** | **1.72E-01** | **19.1%** | **0.926** | **7.58E-03** | **0.8%** | **0.269** | **2.26E-03** | **0.3%** | **0.182** | **20.2%** |
| 1.8a | 142 | 1.3371 | 74.3% | 7.97 | 0.0652 | 3.6% | <LLQ [b] | <LLQ [b] | NA | 1.4023 | 77.9% |
| 1.8b | 145 | 1.3653 | 75.9% | 8.03 | 0.0657 | 3.7% | <LLQ [b] | <LLQ [b] | NA | 1.4311 | 79.5% |
| 1.8c | 130 | 1.2241 | 68.0% | 8.26 | 0.0676 | 3.8% | <LLQ [b] | <LLQ [b] | NA | 1.2917 | 71.8% |
| **Mean** | **139** | **1.31E+00** | **72.7%** | **8.09** | **6.62E-02** | **3.7%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **1.38** | **76.4%** |
| **Std. Dev** | **7.94** | **7.47E-02** | **4.2%** | **0.153** | **1.25E-03** | **0.1%** | | | **NA** | **0.0736** | **4.1%** |
| 3.5a | 313 | 2.9473 | 84.2% | 8.69 | 0.0711 | 2.0% | 0.490 | 0.0041 | 0.1% | 3.0225 | 86.4% |
| 3.5b | 293 | 2.7589 | 78.8% | 10.5 | 0.0859 | 2.5% | 0.601 | 0.0050 | 0.1% | 2.8499 | 81.4% |
| 3.5c | 246 | 2.3164 | 66.2% | 8.28 | 0.0678 | 1.9% | <LLQ [b] | <LLQ [b] | NA | 2.3841 | 68.1% |
| **Mean** | **284** | **2.67E+00** | **76.4%** | **9.16** | **7.49E-02** | **2.1%** | **0.486** | **4.03E-03** | **0.1%** | **2.75** | **78.6%** |
| **Std. Dev** | **34.4** | **3.24E-01** | **9.3%** | **1.18** | **9.67E-03** | **0.3%** | **0.117** | **1.00E-03** | **0.0%** | **0.330** | **9.4%** |
| 7a | 611 | 5.7533 | 82.2% | 12.4 | 0.1015 | 1.4% | 0.401 | 0.0033 | 0.0% | 5.8581 | 83.7% |
| 7b | NS | NA | NA | 11.8 | 0.0966 | 1.4% | 0.473 | 0.0039 | 0.1% | 0.1005 | 1.4% |
| 7c | 538 | 5.0659 | 72.4% | 8.48 | 0.0694 | 1.0% | 0.511 | 0.0043 | 0.1% | 5.1396 | 73.4% |
| **Mean** | **575** | **5.41E+00** | **77.3%** | **10.9** | **8.91E-02** | **1.3%** | **0.462** | **3.84E-03** | **0.1%** | **3.70** | **52.8%** |
| **Std. Dev** | **NA** | **NA** | **NA** | **2.11** | **1.73E-02** | **0.2%** | **0.0559** | **4.65E-04** | **0.01%** | **3.137** | **44.8%** |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 2. Individual Values from GC/MS Analysis of Lung Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone

| Treatment | Mouse Lung Incubations | | | Rat Lung Incubations | | | Human Lung Incubations | | |
|---|---|---|---|---|---|---|---|---|---|
| | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) | EB (µg/mL) | 1-PE (µg/mL) | AcPh (µg/mL) |
| Control no microsomes | 34 9 | <LLQ [b] | <LLQ [c] | | | | | | |
| Control no substrate | <LLQ [a] | <LLQ [b] | <LLQ [c] | <LLQ [a] | <LLQ [b] | <LLQ [c] | <LLQ [a] | <LLQ [b] | 0 452 |
| 0 45 no NADPH | 34 1 | <LLQ [b] | <LLQ [c] | 36 4 | <LLQ [b] | <LLQ [c] | 27 2 | <LLQ [b] | <LLQ [c] |
| 1 8 no NADPH | 127 | <LLQ [b] | <LLQ [c] | 141 | <LLQ [b] | <LLQ [c] | 112 | <LLQ [b] | <LLQ [c] |
| 7 no NADPH | 473 | <LLQ [b] | <LLQ [c] | 585 | <LLQ [b] | <LLQ [c] | 542 | <LLQ [b] | <LLQ [c] |
| 0 22a | 8.84 | 15 3 | 1.86 | 16.0 | 8 27 | 0 371 | 20.9 | <LLQ [b] | <LLQ [c] |
| 0 22b | 7 79 | 15 2 | 1 79 | 15 1 | 8 11 | <LLQ [c] | 19 5 | <LLQ [b] | <LLQ [c] |
| 0 22c | 6.97 | 15.9 | 1.90 | 14.2 | 8.02 | 0.369 | 22.1 | <LLQ [b] | <LLQ [c] |
| Average: | **7 87** | **15.5** | **1.85** | **15.1** | **8.13** | **0.369** | **20.8** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 11.9% | 2.45% | 3.01% | 5.96% | 1.56% | 0.54% | 6.25% | | |
| 0.45a | 15 4 | 24.8 | 2 42 | 25 2 | 9 11 | <LLQ [c] | 38 7 | <LLQ [b] | <LLQ [c] |
| 0.45b | 14.8 | 30.3 | 2 77 | 25 1 | 9.04 | <LLQ [c] | 42 3 | <LLQ [b] | <LLQ [c] |
| 0.45c | 15.3 | 30.2 | 2.65 | 24.3 | 9.24 | <LLQ [c] | 40.6 | <LLQ [b] | <LLQ [c] |
| Average: | **15.2** | **28.4** | **2.61** | **24.9** | **9.13** | **<LLQ [c]** | **40.5** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 2.12% | 11.07% | 6.81% | 1.98% | 1.11% | | 4.44% | | |
| 0.9a | 43 8 | 45.6 | 2.85 | 51.0 | 9.74 | <LLQ [c] | 100 | <LLQ [b] | <LLQ [c] |
| 0.9b | 43.0 | 47 9 | 2.86 | 58 7 | 10.1 | <LLQ [c] | 100 | <LLQ [b] | <LLQ [c] |
| 0.9c | 45.2 | 47.6 | 2.43 | 55.8 | 9.52 | <LLQ [c] | 106 | <LLQ [b] | <LLQ [c] |
| Average: | **44.0** | **47.0** | **2.71** | **55.2** | **9.79** | **<LLQ [c]** | **102** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 2.53% | 2.66% | 9.05% | 7.05% | 2.99% | | 3.40% | | |
| 1.8a | 131 | 53.6 | 2 51 | 1 12 | 10.0 | <LLQ [c] | 202 | <LLQ [b] | <LLQ [c] |
| 1.8b | 127 | 52.6 | 2.51 | 1 14 | 11 4 | <LLQ [c] | 205 | <LLQ [b] | <LLQ [c] |
| 1 8c | 126 | 50.6 | 2.16 | 125 | 10.0 | <LLQ [c] | 210 | <LLQ [b] | <LLQ [c] |
| Average: | **128** | **52.3** | **2.39** | **117** | **10.5** | **<LLQ [c]** | **206** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 2.07% | 2.92% | 8.44% | 5.98% | 7.72% | | 1.97% | | |
| 3 5a | 281 | 52 8 | 2.61 | 233 | 10.6 | <LLQ [c] | 273 | <LLQ [b] | <LLQ [c] |
| 3 5b | 281 | 52 8 | 2.56 | 247 | 11.0 | <LLQ [c] | 316 | <LLQ [b] | <LLQ [c] |
| 3 5c | 292 | 49.1 | 2.20 | 255 | 10.7 | <LLQ [c] | 295 | <LLQ [b] | <LLQ [c] |
| Average: | **285** | **51.6** | **2.46** | **245** | **10.8** | **<LLQ [c]** | **295** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 2.23% | 4.14% | 9.11% | 4.55% | 1.93% | | 7.30% | | |
| 7a | 707 | 58.6 | 2.81 | 574 | 10.2 | <LLQ [c] | 342 | <LLQ [b] | <LLQ [c] |
| 7b | 663 | 51.8 | 2 16 | 584 | 11 2 | <LLQ [c] | 598 | <LLQ [b] | <LLQ [c] |
| 7c | 660 | 51.1 | 2.30 | 526 | 10.3 | <LLQ [c] | 490 | <LLQ [b] | <LLQ [c] |
| Average: | **677** | **53.8** | **2.42** | **561** | **10.6** | **<LLQ [c]** | **477** | **<LLQ [b]** | **<LLQ [c]** |
| %RSD: | 3.89% | 7.70% | 14.1% | 5.52% | 5.21% | | 27.0% | | |

[a] <LLQ = less than lowest level quantitated = 2.32 µg ethylbenzene/mL
[b] <LLQ = less than lowest level quantitated = 0.360 µg 1-phenylethanol/mL
[c] <LLQ = less than lowest level quantitated = 0.367 µg acetophenone/mL

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 2 Individual Values from GC/MS Analysis of Lung Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Mouse Lung Incubations (from 10/12/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) | EB | | | 1-PE | | | AcPh | | | EB+1-PE+AcPh (mass balance) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (µmole / vessel) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µmole) | (%) |
| 0.22a | 8.84 | 0.0832 | 37.8% | 15.3 | 0.1252 | 56.9% | 1.86 | 0.0155 | 7.0% | 0.2239 | 101.8% |
| 0.22b | 7.79 | 0.0734 | 33.3% | 15.2 | 0.1244 | 56.5% | 1.79 | 0.0149 | 6.8% | 0.2126 | 96.7% |
| 0.22c | 6.97 | 0.0656 | 29.8% | 15.9 | 0.1301 | 59.1% | 1.90 | 0.0158 | 7.2% | 0.2116 | 96.2% |
| **Mean** | **7.87** | **7.41E-02** | **33.7%** | **15.5** | **1.27E-01** | **57.5%** | **1.85** | **1.54E-02** | **7.0%** | **0.216** | **98.2%** |
| **Std. Dev** | **0.937** | **8.83E-03** | **4.0%** | **0.379** | **3.10E-03** | **1.4%** | **0.0557** | **4.63E-04** | **0.2%** | **0.0068** | **3.1%** |
| 0.45a | 15.4 | 0.1450 | 32.2% | 24.8 | 0.2029 | 45.1% | 2.42 | 0.0201 | 4.5% | 0.3681 | 81.8% |
| 0.45b | 14.8 | 0.1394 | 31.0% | 30.3 | 0.2480 | 55.1% | 2.77 | 0.0230 | 5.1% | 0.4104 | 91.2% |
| 0.45c | 15.3 | 0.1441 | 32.0% | 30.2 | 0.2471 | 54.9% | 2.65 | 0.0220 | 4.9% | 0.4133 | 91.8% |
| **Mean** | **15.2** | **1.43E-01** | **31.7%** | **28.4** | **2.33E-01** | **51.7%** | **2.61** | **2.17E-02** | **4.8%** | **0.397** | **88.3%** |
| **Std. Dev** | **0.321** | **3.03E-03** | **0.7%** | **3.15** | **2.58E-02** | **5.7%** | **0.178** | **1.48E-03** | **0.3%** | **0.0253** | **5.6%** |
| 0.9a | 43.8 | 0.4124 | 45.8% | 45.6 | 0.3732 | 41.5% | 2.85 | 0.0237 | 2.6% | 0.8093 | 89.9% |
| 0.9b | 43.0 | 0.4049 | 45.0% | 47.9 | 0.3920 | 43.6% | 2.86 | 0.0238 | 2.6% | 0.8207 | 91.2% |
| 0.9c | 45.2 | 0.4256 | 47.3% | 47.6 | 0.3895 | 43.3% | 2.43 | 0.0202 | 2.2% | 0.8354 | 92.8% |
| **Mean** | **44.0** | **4.14E-01** | **46.0%** | **47.0** | **3.85E-01** | **42.8%** | **2.71** | **2.26E-02** | **2.5%** | **0.82** | **91.3%** |
| **Std. Dev** | **1.11** | **1.05E-02** | **1.2%** | **1.25** | **1.02E-02** | **1.1%** | **0.245** | **2.04E-03** | **0.2%** | **0.013** | **1.5%** |
| 1.8a | 131 | 1.2335 | 68.5% | 53.6 | 0.4386 | 24.4% | 2.51 | 0.0209 | 1.2% | 1.6930 | 94.1% |
| 1.8b | 127 | 1.1959 | 66.4% | 52.6 | 0.4304 | 23.9% | 2.51 | 0.0209 | 1.2% | 1.6472 | 91.5% |
| 1.8c | 126 | 1.1864 | 65.9% | 50.6 | 0.4141 | 23.0% | 2.16 | 0.0180 | 1.0% | 1.6185 | 89.9% |
| **Mean** | **128** | **1.21E+00** | **67.0%** | **52.3** | **4.28E-01** | **23.8%** | **2.39** | **1.99E-02** | **1.1%** | **1.653** | **91.8%** |
| **Std. Dev** | **2.65** | **2.49E-02** | **1.4%** | **1.53** | **1.25E-02** | **0.7%** | **0.202** | **1.68E-03** | **0.1%** | **0.0376** | **2.1%** |
| 3.5a | 281 | 2.6460 | 75.6% | 52.8 | 0.4321 | 12.3% | 2.61 | 0.0217 | 0.6% | 3.0997 | 88.6% |
| 3.5b | 281 | 2.6460 | 75.6% | 52.8 | 0.4321 | 12.3% | 2.56 | 0.0213 | 0.6% | 3.0993 | 88.6% |
| 3.5c | 292 | 2.7495 | 78.6% | 49.1 | 0.4018 | 11.5% | 2.20 | 0.0183 | 0.5% | 3.1696 | 90.6% |
| **Mean** | **285** | **2.68E+00** | **76.6%** | **51.6** | **4.22E-01** | **12.1%** | **2.46** | **2.04E-02** | **0.6%** | **3.123** | **89.2%** |
| **Std. Dev** | **6.35** | **5.98E-02** | **1.7%** | **2.14** | **1.75E-02** | **0.5%** | **0.224** | **1.86E-03** | **0.1%** | **0.040** | **1.2%** |
| 7a | 707 | 6.6573 | 95.1% | 58.6 | 0.4795 | 6.9% | 2.81 | 0.0234 | 0.3% | 7.1602 | 102.3% |
| 7b | 663 | 6.2429 | 89.2% | 51.8 | 0.4239 | 6.1% | 2.16 | 0.0180 | 0.3% | 6.6848 | 95.5% |
| 7c | 660 | 6.2147 | 88.8% | 51.1 | 0.4182 | 6.0% | 2.30 | 0.0191 | 0.3% | 6.6520 | 95.0% |
| **Mean** | **677** | **6.37E+00** | **91.0%** | **53.8** | **4.41E-01** | **6.3%** | **2.42** | **2.02E-02** | **0.3%** | **6.832** | **97.6%** |
| **Std. Dev** | **26.3** | **2.48E-01** | **3.5%** | **4.14** | **3.39E-02** | **0.5%** | **0.342** | **2.85E-03** | **0.0%** | **0.284** | **4.1%** |
| | | | | | | | | | | Mean % of Nominal | **92.7%** |
| | | | | | | | | | | (thus, use nominal concentrations for initial substrate) | |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 2 Individual Values from GC/MS Analysis of Lung Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Rat Lung Incubations (from 10/12/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) | EB | | | 1-PE | | | AcPh | | | EB+1-PE+AcPh (mass balance) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (µmole / vessel) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µg/mL) | (µmole) | (%Conv) | (µmole) | (%) |
| 0.22a | 16.0 | 0.1507 | 68.5% | 8.27 | 0.0677 | 30.8% | 0.371 | 0.0031 | 1.4% | 0.2214 | 100.6% |
| 0.22b | 15.1 | 0.1422 | 64.6% | 8.11 | 0.0664 | 30.2% | <LLQ [a] | <LLQ [b] | NA | 0.2086 | 94.8% |
| 0.22c | 14.2 | 0.1337 | 60.8% | 8.02 | 0.0656 | 29.8% | 0.369 | 0.0031 | 1.4% | 0.2024 | 92.0% |
| **Mean** | **15.1** | **1.42E-01** | **64.6%** | **8.13** | **6.66E-02** | **30.3%** | **0.369** | **3.05E-03** | **1.4%** | **0.211** | **95.8%** |
| **Std. Dev** | **0.900** | **8.47E-03** | **3.9%** | **0.127** | **1.04E-03** | **0.5%** | **0.00200** | **4.59E-05** | **0.02%** | **0.0097** | **4.4%** |
| 0.45a | 25.2 | 0.2373 | 52.7% | 9.11 | 0.0745 | 16.6% | <LLQ [b] | <LLQ [b] | NA | 0.3118 | 69.3% |
| 0.45b | 25.1 | 0.2363 | 52.5% | 9.04 | 0.0740 | 16.4% | <LLQ [b] | <LLQ [b] | NA | 0.3103 | 69.0% |
| 0.45c | 24.3 | 0.2288 | 50.8% | 9.24 | 0.0756 | 16.8% | <LLQ [b] | <LLQ [b] | NA | 0.3044 | 67.7% |
| **Mean** | **24.9** | **2.34E-01** | **52.0%** | **9.13** | **7.47E-02** | **16.6%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.309** | **68.6%** |
| **Std. Dev** | **0.493** | **4.64E-03** | **1.0%** | **0.101** | **8.31E-04** | **0.2%** | | | **NA** | **0.0039** | **0.9%** |
| 0.9a | 51.0 | 0.4802 | 53.4% | 9.74 | 0.0797 | 8.9% | <LLQ [b] | <LLQ [b] | NA | 0.5599 | 62.2% |
| 0.9b | 58.7 | 0.5527 | 61.4% | 10.1 | 0.0827 | 9.2% | <LLQ [b] | <LLQ [b] | NA | 0.6354 | 70.6% |
| 0.9c | 55.8 | 0.5254 | 58.4% | 9.52 | 0.0779 | 8.7% | <LLQ [b] | <LLQ [b] | NA | 0.6033 | 67.0% |
| **Mean** | **55.2** | **5.19E-01** | **57.7%** | **9.79** | **8.01E-02** | **8.9%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.600** | **66.8%** |
| **Std. Dev** | **3.89** | **3.66E-02** | **4.1%** | **0.293** | **2.40E-03** | **0.3%** | | | **NA** | **0.038** | **2.5%** |
| 1.8a | 112 | 1.0546 | 58.6% | 10.0 | 0.0818 | 4.5% | <LLQ [b] | <LLQ [b] | NA | 1.1364 | 63.1% |
| 1.8b | 114 | 1.0734 | 59.6% | 11.4 | 0.0933 | 5.2% | <LLQ [b] | <LLQ [b] | NA | 1.1667 | 64.8% |
| 1.8c | 125 | 1.1770 | 65.4% | 10.0 | 0.0818 | 4.5% | <LLQ [b] | <LLQ [b] | NA | 1.2589 | 69.9% |
| **Mean** | **117** | **1.10E+00** | **61.2%** | **10.5** | **8.57E-02** | **4.8%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **1.19** | **66.0%** |
| **Std. Dev** | **7.00** | **6.59E-02** | **3.7%** | **0.808** | **6.61E-03** | **0.4%** | | | **NA** | **0.064** | **3.5%** |
| 3.5a | 233 | 2.1940 | 62.7% | 10.6 | 0.0867 | 2.5% | <LLQ [b] | <LLQ [b] | NA | 2.2807 | 65.2% |
| 3.5b | 247 | 2.3258 | 66.5% | 11.0 | 0.0900 | 2.6% | <LLQ [b] | <LLQ [b] | NA | 2.4158 | 69.0% |
| 3.5c | 255 | 2.4011 | 68.6% | 10.7 | 0.0876 | 2.5% | <LLQ [b] | <LLQ [b] | NA | 2.4887 | 71.1% |
| **Mean** | **245** | **2.31E+00** | **65.9%** | **10.8** | **8.81E-02** | **2.5%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **2.40** | **68.4%** |
| **Std. Dev** | **11.1** | **1.05E-01** | **3.0%** | **0.208** | **1.70E-03** | **0.0%** | | | **NA** | **0.106** | **3.0%** |
| 7a | 574 | 5.4049 | 77.2% | 10.2 | 0.0835 | 1.2% | <LLQ [b] | <LLQ [b] | NA | 5.4884 | 78.4% |
| 7b | 584 | 5.4991 | 78.6% | 11.2 | 0.0917 | 1.3% | <LLQ [b] | <LLQ [b] | NA | 5.5907 | 79.9% |
| 7c | 526 | 4.9529 | 70.8% | 10.3 | 0.0843 | 1.2% | <LLQ [b] | <LLQ [b] | NA | 5.0372 | 72.0% |
| **Mean** | **561** | **5.29E+00** | **75.5%** | **10.6** | **8.65E-02** | **1.2%** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **5.37** | **76.7%** |
| **Std. Dev** | **31.0** | **2.92E-01** | **4.2%** | **0.551** | **4.51E-03** | **0.1%** | | | **NA** | **0.295** | **4.2%** |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 2. Individual Values from GC/MS Analysis of Lung Microsome Incubation Solutions for Ethylbenzene, 1-Phenylethanol, and Acetophenone (continued)

Human Lung Incubations (from 10/12/06); 30 min incubation; 1 mg protein per incubate

| Substrate (EB) (µmole / vessel) | EB (µg/mL) | EB (µmole) | EB (%Conv) | 1-PE (µg/mL) | 1-PE (µmole) | 1-PE (%Conv) | AcPh (µg/mL) | AcPh (µmole) | AcPh (%Conv) | EB+1-PE+AcPh (mass balance) (µmole) | EB+1-PE+AcPh (mass balance) (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.22a | 20 9 | 0 1968 | 89 5% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 1968 | 89 5% |
| 0 22b | 19 5 | 0 1836 | 83 5% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 1836 | 83 5% |
| 0.22c | 22 1 | 0 2081 | 94 6% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 2081 | 94 6% |
| **Mean** | **20.8** | **1 96E-01** | **89.2%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.196** | **89.2%** |
| **Std. Dev** | **1.30** | **1.23E-02** | **5.6%** | | | **NA** | | | **NA** | **0.0123** | **5.6%** |
| 0.45a | 38 7 | 0.3644 | 81 0% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 3644 | 81 0% |
| 0.45b | 42 3 | 0.3983 | 88 5% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 3983 | 88 5% |
| 0.45c | 40.6 | 0 3823 | 85 0% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 3823 | 85.0% |
| **Mean** | **40.5** | **3.82E-01** | **84.8%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.382** | **84.8%** |
| **Std. Dev** | **1.80** | **1.70E-02** | **3.8%** | | | **NA** | | | **NA** | **0.0170** | **3.8%** |
| 0.9a | 100 | 0.9416 | 105% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 9416 | 104.6% |
| 0 9b | 100 | 0.9416 | 105% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 9416 | 104.6% |
| 0.9c | 106 | 0 9981 | 111% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 0 9981 | 110 9% |
| **Mean** | **102** | **9.60E-01** | **107%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **0.960** | **107.8%** |
| **Std. Dev** | **3.46** | **3.26E-02** | **3.6%** | | | **NA** | | | **NA** | **0.033** | **4.4%** |
| 1 8a | 202 | 1 9021 | 106% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 1 9021 | 105 7% |
| 1 8b | 205 | 1 9303 | 107% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 1 9303 | 107 2% |
| 1 8c | 210 | 1 9774 | 110% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 1 9774 | 109 9% |
| **Mean** | **206** | **1.94E+00** | **108%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **1.94** | **107.6%** |
| **Std. Dev** | **4.04** | **3.81E-02** | **2.1%** | | | **NA** | | | **NA** | **0.038** | **2.1%** |
| 3 5a | 273 | 2 5706 | 73 4% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 2 5706 | 73 4% |
| 3 5b | 316 | 2 9755 | 85 0% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 2 9755 | 85 0% |
| 3 5c | 295 | 2 7778 | 79 4% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 2 7778 | 79 4% |
| **Mean** | **295** | **2.77E+00** | **79.3%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **2.77** | **79.3%** |
| **Std. Dev** | **21.5** | **2.02E-01** | **5.8%** | | | **NA** | | | **NA** | **0.202** | **5.8%** |
| 7a | 342 | 3 2203 | 46.0% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 3 2203 | 46.0% |
| 7b | 598 | 5.6309 | 80 4% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 5.6309 | 80 4% |
| 7c | 490 | 4.6139 | 65 9% | <LLQ [a] | <LLQ [a] | NA | <LLQ [b] | <LLQ [b] | NA | 4.6139 | 65 9% |
| **Mean** | **477** | **4.49E+00** | **64.1%** | **<LLQ [a]** | **<LLQ [a]** | **NA** | **<LLQ [b]** | **<LLQ [b]** | **NA** | **4.49** | **64.1%** |
| **Std. Dev** | **129** | **1.21E+00** | **17.3%** | | | **NA** | | | **NA** | **1.210** | **17.3%** |

[a] <LLQ = less than lowest level quantitated = 0.360 µg (or 0.003 µmole) 1-phenylethanol per incubation vial

[b] <LLQ = less than lowest level quantitated = 0.367 µg (or 0.003 µmole) acetophenone per incubation vial

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 3. Individual Values from HPLC/MRM/MS Analysis of Liver Microsome Incubation Solutions for 2EP-GSH and 4EP-GSH

| | Mouse Liver Incubations | | | | | | Rat Liver Incubations | | | | | | Human Liver Incubations | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2EP-GSH | | | 4EP-GSH | | | 2EP-GSH | | | 4EP-GSH | | | 2EP-GSH | | | 4EP-GSH | | |
| Treatment | (ng/mL) | mM | %Conversion | (ng/mL) | mM | %Conversion | (ng/mL) | mM | %Conversion | (ng/mL) | mM | %Conversion | (ng/mL) | mM | %Conversion | (ng/mL) | mM | %Conversion |
| control no microsomes | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| control no substrate | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | 4.20 | 9.482E-06 | NA | 286474838 | 6.4667E-06 | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.45 no NADPH | 5.81 | 1.31E05 | 0.00291% | <LLQ | <LLQ | NA | 4.74 | 1.07E05 | 0.00238% | <LLQ | <LLQ | NA | 15.7 | 3.54E05 | 0.00787% | <LLQ | <LLQ | NA |
| 1.8 no NADPH | 20.9 | 4.71E05 | 0.00362% | <LLQ | <LLQ | NA | 22.9 | 5.17E05 | 0.00287% | <LLQ | <LLQ | NA | <LLQ | <LLQ | | <LLQ | <LLQ | NA |
| 7 no NADPH | 61.9 | 1.40E04 | 0.00199% | <LLQ | <LLQ | NA | 58.4 | 1.32E04 | 0.00188% | <LLQ | <LLQ | NA | 22.7 | 5.13E05 | 0.000733% | <LLQ | <LLQ | NA |
| 0.22a | 265 | 5.99E04 | 0.272% | 13.6 | 3.08E05 | 0.0140% | 32.0 | 7.23E05 | 0.0329% | 3.62 | 8.18E06 | 0.00372% | 22.5 | 5.09E05 | 0.0231% | 38.6 | 8.70E05 | 0.0396% |
| 0.22b | 299 | 6.75E04 | 0.307% | 18.8 | 4.25E05 | 0.0193% | 24.7 | 5.58E05 | 0.0254% | 4.53 | 1.02E05 | 0.00465% | 22.1 | 5.00E05 | 0.0227% | 39.7 | 8.96E05 | 0.0407% |
| 0.22c | 278 | 6.28E04 | 0.286% | 17.3 | 3.90E05 | 0.0177% | 23.0 | 5.18E05 | 0.0236% | 3.69 | 8.33E06 | 0.00378% | 25.4 | 5.73E05 | 0.0261% | 38.0 | 8.57E05 | 0.0389% |
| Average: | **281** | **6.34E-04** | **0.288%** | **16.6** | **3.74E-05** | **0.0170%** | **26.6** | **6.00E-05** | **0.0273%** | **3.95** | **8.91E-06** | **0.00405%** | **23.4** | **5.27E-05** | **0.0240%** | **38.7** | **8.74E-05** | **0.0397%** |
| %RSD: | 6.07% | 6.07% | 6.07% | 16.0% | 16.0% | 16.0% | 18.1% | 18.1% | 18.1% | 12.8% | 12.8% | 12.8% | 7.59% | 7.59% | 7.59% | 2.27% | 2.27% | 2.27% |
| 0.45a | 251 | 5.66E04 | 0.126% | 14.7 | 3.31E05 | 0.00737% | 29.2 | 6.59E05 | 0.0147% | <LLQ | <LLQ | NA | 36.0 | 8.13E05 | 0.0181% | 37.9 | 8.55E05 | 0.0190% |
| 0.45b | 238 | 5.37E04 | 0.119% | 14.1 | 3.19E05 | 0.00709% | 27.0 | 6.09E05 | 0.0135% | <LLQ | <LLQ | NA | 24.7 | 5.56E05 | 0.0124% | 34.1 | 7.69E05 | 0.0171% |
| 0.45c | 252 | 5.69E04 | 0.126% | 12.6 | 2.85E05 | 0.00633% | 21.6 | 4.88E05 | 0.0108% | 1.68 | 3.79E06 | 0.000843% | 19.7 | 4.44E05 | 0.0099% | 26.1 | 5.88E05 | 0.0131% |
| Average: | **247** | **5.57E-04** | **0.124%** | **13.8** | **3.12E-05** | **0.00693%** | **25.9** | **5.85E-05** | **0.0130%** | **1.65** | **3.73E-06** | **0.000829%** | **26.8** | **6.04E-05** | **0.0134%** | **32.7** | **7.38E-05** | **0.0164%** |
| %RSD: | 3.20% | 3.20% | 3.20% | 7.76% | 7.76% | 7.76% | 15.1% | 15.1% | 15.1% | 1.4% | 1.4% | 1.4% | 31.3% | 31.3% | 31.3% | 18.5% | 18.5% | 18.5% |
| 0.9a | 227 | 5.12E04 | 0.0569% | 12.6 | 2.85E05 | 0.00317% | 39.4 | 8.90E05 | 0.00989% | 2.00 | 4.51E06 | 0.000501% | 45.9 | 1.04E04 | 0.0115% | 24.7 | 5.58E05 | 0.00620% |
| 0.9b | 201 | 4.55E04 | 0.0505% | 9.30 | 2.10E05 | 0.00233% | 36.1 | 8.15E05 | 0.00905% | 1.68 | 3.80E06 | 0.000422% | 29.8 | 6.72E05 | 0.00747% | 25.7 | 5.80E05 | 0.00644% |
| 0.9c | 184 | 4.15E04 | 0.0461% | 7.92 | 1.79E05 | 0.00199% | 26.2 | 5.91E05 | 0.00657% | <LLQ | <LLQ | NA | 31.0 | 7.00E05 | 0.00777% | 27.5 | 6.21E05 | 0.00690% |
| Average: | **204** | **4.61E-04** | **0.0512%** | **9.95** | **2.25E-05** | **0.00250%** | **33.9** | **7.65E-05** | **0.00850%** | **1.77** | **4.00E-06** | **0.000445%** | **35.6** | **8.03E-05** | **0.00892%** | **26.0** | **5.86E-05** | **0.00651%** |
| %RSD: | 10.6% | 10.6% | 10.6% | 24.4% | 24.4% | 24.4% | 20.3% | 20.3% | 20.3% | 11.1% | 11.1% | 11.1% | 25.2% | 25.2% | 25.2% | 5.48% | 5.48% | 5.48% |
| 1.8a | 166 | 3.74E04 | 0.0208% | 5.92 | 1.34E05 | 0.000742% | 42.3 | 9.56E05 | 0.00531% | 1.75 | 3.94E06 | 0.000219% | 66.4 | 1.50E04 | 0.00833% | 20.8 | 4.69E05 | 0.00261% |
| 1.8b | 166 | 3.75E04 | 0.0208% | 5.38 | 1.21E05 | 0.000674% | 41.1 | 9.27E05 | 0.00515% | <LLQ | <LLQ | NA | 61.3 | 1.38E04 | 0.00769% | 19.8 | 4.47E05 | 0.00248% |
| 1.8c | 156 | 3.53E04 | 0.0196% | 6.43 | 1.45E05 | 0.000807% | 43.2 | 9.76E05 | 0.00542% | <LLQ | <LLQ | NA | 56.5 | 1.27E04 | 0.00708% | 16.0 | 3.60E05 | 0.00200% |
| Average: | **163** | **3.67E-04** | **0.0204%** | **5.91** | **1.33E-05** | **0.000741%** | **42.2** | **9.53E-05** | **0.00529%** | **1.68** | **3.78E-06** | **0.000210%** | **61.4** | **1.39E-04** | **0.00770%** | **18.8** | **4.25E-05** | **0.00236%** |
| %RSD: | 3.34% | 3.34% | 3.34% | 8.96% | 8.96% | 8.96% | 2.60% | 2.60% | 2.60% | 3.8% | 3.8% | 3.8% | 8.14% | 8.14% | 8.14% | 13.5% | 13.5% | 13.5% |
| 3.5a | 177 | 4.00E04 | 0.0114% | 4.29 | 9.69E06 | 0.000277% | 64.9 | 1.47E04 | 0.00419% | <LLQ | <LLQ | NA | 126 | 2.86E04 | 0.00816% | 13.2 | 2.99E05 | 0.000853% |
| 3.5b | 148 | 3.33E04 | 0.00952% | 4.14 | 9.34E06 | 0.000267% | 63.5 | 1.43E04 | 0.00409% | 4.07 | 9.19E06 | 0.000263% | 119 | 2.68E04 | 0.00765% | 12.0 | 2.71E05 | 0.000774% |
| 3.5c | 139 | 3.14E04 | 0.00897% | 5.81 | 1.31E05 | 0.000375% | 76.5 | 1.73E04 | 0.00493% | <LLQ | <LLQ | NA | 72.0 | 1.63E04 | 0.00465% | 1.81 | 4.09E06 | 0.000117% |
| Average: | **155** | **3.49E-04** | **0.00997%** | **4.75** | **1.07E-05** | **0.000306%** | **68.3** | **1.54E-04** | **0.00440%** | **2.45** | **5.53E-06** | **0.000158%** | **106** | **2.39E-04** | **0.00682%** | **9.01** | **2.03E-05** | **0.000581%** |
| %RSD: | 12.9% | 12.9% | 12.9% | 19.5% | 19.5% | 19.5% | 10.5% | 10.5% | 10.5% | 57.3% | 57.3% | 57.3% | 27.8% | 27.8% | 27.8% | 69.5% | 69.5% | 69.5% |
| 7a | 223 | 5.03E04 | 0.00719% | 3.34 | 7.54E06 | 0.000108% | 117 | 2.63E04 | 0.00376% | 4.41 | 9.95E06 | 0.000142% | 136 | 3.06E04 | 0.00437% | 14.3 | 3.22E05 | 0.000460% |
| 7b | 167 | 3.77E04 | 0.00539% | 3.51 | 7.93E06 | 0.000113% | 127 | 2.86E04 | 0.00409% | 5.07 | 1.14E05 | 0.000163% | 116 | 2.62E04 | 0.00375% | 12.2 | 2.74E05 | 0.000392% |
| 7c | 185 | 4.18E04 | 0.00598% | 2.91 | 6.58E06 | 0.0000939% | 128 | 2.90E04 | 0.00414% | 5.13 | 1.16E05 | 0.000166% | 106 | 2.39E04 | 0.00342% | 8.9 | 2.00E05 | 0.000286% |
| Average: | **192** | **4.33E-04** | **0.00618%** | **3.26** | **7.35E-06** | **0.000105%** | **124** | **2.80E-04** | **0.00399%** | **4.87** | **1.10E-05** | **0.000157%** | **119** | **2.69E-04** | **0.00385%** | **11.8** | **2.66E-05** | **0.000379%** |
| %RSD: | 14.8% | 14.8% | 14.8% | 9.51% | 9.51% | 9.51% | 5.14% | 5.14% | 5.14% | 8.22% | 8.22% | 8.22% | 12.6% | 12.6% | 12.6% | 23.2% | 23.2% | 23.2% |

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix Table 4. Individual Values from HPLC/MRM/MS Analysis of Lung Microsome Incubation Solutions for 2EP-GSH and 4EP-GSH

| | Mouse Lung Incubations | | | | | | Rat Lung Incubations | | | | | | Human Lung Incubations | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2EP-GSH | | | 4EP-GSH | | | 2EP-GSH | | | 4EP-GSH | | | 2EP-GSH | | | 4EP-GSH | | |
| Treatment | (ng/mL) | mM | % Conversion | (ng/mL) | mM | % Conversion | (ng/mL) | mM | % Conversion | (ng/mL) | mM | % Conversion | (ng/mL) | mM | % Conversion | (ng/mL) | mM | % Conversion |
| Control no microsomes | NA | NA | NA | NA | NA | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | NA | NA | NA | NA | NA | NA |
| Control no substrate | 3.64 | 8.21E-06 | NA | <LLQ | <LLQ | NA | 2.55 | 5.7517E-06 | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.45 no NADPH | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 1.8 no NADPH | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 7 no NADPH | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.22a | 536 | 1.21E-03 | 0.550% | 17.3 | 3.91E-05 | 0.0178% | 52.3 | 1.18E-04 | 0.0537% | 10.7 | 2.41E-05 | 0.0110% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.22b | 542 | 1.22E-03 | 0.556% | 16.0 | 3.61E-05 | 0.0164% | 60.0 | 1.35E-04 | 0.0616% | 12.6 | 2.84E-05 | 0.0129% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.22c | 533 | 1.20E-03 | 0.547% | 18.9 | 4.27E-05 | 0.0194% | 59.3 | 1.34E-04 | 0.0609% | 11.9 | 2.69E-05 | 0.0122% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **537** | **1.21E-03** | **0.551%** | **17.4** | **3.93E-05** | **0.0179%** | **57.2** | **1.29E-04** | **0.0587%** | **11.7** | **2.65E-05** | **0.0120%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 0.84% | 0.84% | 0.84% | 8.43% | 8.43% | 8.43% | 7.42% | 7.42% | 7.42% | 8.13% | 8.13% | 8.13% | NA | NA | NA | NA | NA | NA |
| 0.45a | 510 | 1.15E-03 | 0.256% | 24.9 | 5.62E-05 | 0.0125% | 47.0 | 1.06E-04 | 0.0236% | 8.80 | 1.99E-05 | 0.00441% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.45b | 443 | 1.00E-03 | 0.222% | 27.5 | 6.20E-05 | 0.0138% | 45.4 | 1.02E-04 | 0.0228% | 8.54 | 1.93E-05 | 0.00428% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.45c | 467 | 1.05E-03 | 0.234% | 24.5 | 5.54E-05 | 0.0123% | 42.0 | 9.47E-05 | 0.0210% | 8.63 | 1.95E-05 | 0.00433% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **473** | **1.07E-03** | **0.237%** | **25.6** | **5.79E-05** | **0.0129%** | **44.8** | **1.01E-04** | **0.0225%** | **8.65** | **1.95E-05** | **0.00434%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 7.18% | 7.18% | 7.18% | 6.23% | 6.23% | 6.23% | 5.71% | 5.71% | 5.71% | 1.51% | 1.51% | 1.51% | NA | NA | NA | NA | NA | NA |
| 0.9a | 232 | 5.25E-04 | 0.0583% | 18.5 | 4.17E-05 | 0.00463% | 32.7 | 7.37E-05 | 0.00819% | 6.45 | 1.46E-05 | 0.00162% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.9b | 235 | 5.31E-04 | 0.0590% | 19.8 | 4.47E-05 | 0.00496% | 33.5 | 7.57E-05 | 0.00841% | 6.25 | 1.41E-05 | 0.00157% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 0.9c | 190 | 4.28E-04 | 0.0475% | 17.2 | 3.88E-05 | 0.00431% | 35.5 | 8.00E-05 | 0.00889% | 6.89 | 1.56E-05 | 0.00173% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **219** | **4.94E-04** | **0.0549%** | **18.5** | **4.17E-05** | **0.00463%** | **33.9** | **7.65E-05** | **0.00850%** | **6.53** | **1.47E-05** | **0.00164%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 11.7% | 11.7% | 11.7% | 7.08% | 7.08% | 7.08% | 4.22% | 4.22% | 4.22% | 5.07% | 5.07% | 5.07% | NA | NA | NA | NA | NA | NA |
| 1.8a | 124 | 2.80E-04 | 0.0156% | 11.0 | 2.49E-05 | 0.00138% | 25.5 | 5.75E-05 | 0.00320% | 4.76 | 1.07E-05 | 0.000596% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 1.8b | 142 | 3.20E-04 | 0.0178% | 12.5 | 2.82E-05 | 0.00156% | 26.2 | 5.90E-05 | 0.00328% | 5.00 | 1.13E-05 | 0.000626% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 1.8c | 123 | 2.78E-04 | 0.0155% | 9.72 | 2.19E-05 | 0.00122% | 25.7 | 5.79E-05 | 0.00322% | 5.36 | 1.21E-05 | 0.000672% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **130** | **2.93E-04** | **0.0163%** | **11.1** | **2.50E-05** | **0.00139%** | **25.8** | **5.82E-05** | **0.00323%** | **5.04** | **1.14E-05** | **0.000632%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 8.13% | 8.13% | 8.13% | 12.5% | 12.5% | 12.5% | 1.33% | 1.33% | 1.33% | 6.03% | 6.03% | 6.03% | NA | NA | NA | NA | NA | NA |
| 3.5a | 115 | 2.59E-04 | 0.00740% | 9.87 | 2.23E-05 | 0.000637% | 24.9 | 5.62E-05 | 0.00160% | 4.53 | 1.02E-05 | 0.000292% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 3.5b | 113 | 2.55E-04 | 0.00729% | 9.61 | 2.17E-05 | 0.000620% | 21.0 | 4.73E-05 | 0.00135% | 3.87 | 8.73E-06 | 0.000250% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 3.5c | 95.6 | 2.16E-04 | 0.00617% | 8.09 | 1.83E-05 | 0.000522% | 21.8 | 4.92E-05 | 0.00140% | 3.50 | 7.90E-06 | 0.000226% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **108** | **2.43E-04** | **0.00695%** | **9.19** | **2.07E-05** | **0.000593%** | **22.5** | **5.09E-05** | **0.00145%** | **3.97** | **8.95E-06** | **0.000256%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 9.83% | 9.83% | 9.83% | 10.5% | 10.5% | 10.5% | 9.18% | 9.18% | 9.18% | 13.2% | 13.2% | 13.2% | NA | NA | NA | NA | NA | NA |
| 7a | 87.3 | 1.97E-04 | 0.00281% | 6.13 | 1.38E-05 | 0.000198% | 18.2 | 4.11E-05 | 0.000588% | 2.71 | 6.12E-06 | 0.0000874% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 7b | 66.6 | 1.50E-04 | 0.00215% | 4.81 | 1.09E-05 | 0.000155% | 17.6 | 3.98E-05 | 0.000569% | 2.95 | 6.66E-06 | 0.0000951% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| 7c | 95.5 | 2.16E-04 | 0.00308% | 7.67 | 1.73E-05 | 0.000247% | 18.5 | 4.19E-05 | 0.000598% | 3.18 | 7.18E-06 | 0.000103% | <LLQ | <LLQ | NA | <LLQ | <LLQ | NA |
| Average: | **83.1** | **1.88E-04** | **0.00268%** | **6.20** | **1.40E-05** | **0.000200%** | **18.1** | **4.09E-05** | **0.000585%** | **2.95** | **6.65E-06** | **0.0000950%** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** | **<LLQ** |
| %RSD: | 18.0% | 18.0% | 18.0% | 23.1% | 23.1% | 23.1% | 2.55% | 2.55% | 2.55% | 7.92% | 7.92% | 7.92% | NA | NA | NA | NA | NA | NA |

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix A Protocol

FINAL PROTOCOL

TOXICOLOGY AND ENVIRONMENTAL RESEARCH AND CONSULTING
THE DOW CHEMICAL COMPANY

# PROTOCOL

1803 BUILDING, MIDLAND, MICHIGAN 48674

TITLE: ETHYLBENZENE: IN VITRO METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

| KEY NUMBERS | | PLANNED DATES | |
|---|---|---|---|
| FILE #: | K-001271-027 | EXPERIMENTAL START: | December 01, 2005 |
| STUDY ID: | 051189 | EXPERIMENTAL TERMINATION: | July 1, 2006 |
| PTR #: | 60213-310-1 | ESTIMATED DRAFT REPORT: | September 1, 2006 |
| CAS #: | 100-41-4 | GLP STUDY: | YES |

SPONSOR: Ethylbenzene Panel
Elizabeth J. Moran, Panel Manager
American Chemistry Council, 1300 Wilson Blvd
Arlington, VA 22209

SPONSOR CONTRACT NO: EB-33.0-met2-Dow

TESTING FACILITY: The Dow Chemical Company
Toxicology & Environmental Research and Consulting
1803 Building
Midland, Michigan 48674

**REQUIRED SIGNATURES:**

STUDY DIRECTOR: R. I. Rick, B.S — 11/14/05 /DATE

LEAD SCIENTIST: S. A. Saghir, M.S P H., Ph D , DABT — 11/14/05 /DATE

MANAGEMENT APPROVAL: R. R. Albee, M.S. — 11-14-05 /DATE

SPONSOR or DESIGNEE: Elizabeth J Moran, Ph.D , EB Panel Manager — 11/8/05 /DATE

**MANAGEMENT ASSIGNED STUDY PERSONNEL:**

Technical Reviewer: M J Bartels, Ph D

**ADDITIONAL STUDY CONTACTS:**

Analytical Chemist: D. A. Markham, B S
Document Management: R S Drury, B S.
Quality Assurance Auditor: B I. Stieve, B.S.
Technologist: A. J. Clark, B S

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix A Protocol (continued)

## A. TITLE

ETHYLBENZENE: IN VITRO METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES PHASE II

## B. INTRODUCTION

### Purpose

The objectives of this study are:

1. Determine the metabolism of 2- and 4-ethylphenol (metabolites of EB) to catechols and hydroquinones by the lung and liver microsomes of three species.
2. Determine the rate of microsomal metabolism of ethylbenzene (EB) to 1-phenylethanol, catechols and hydroquinones. The latter two will be determined by some trapping technique as both of them are highly reactive.

### Test Guidelines

There are no data requirements for this study.

## C. TEST MATERIAL INFORMATION

### Test Material Name

Ethylbenzene

2-ethylphenol; (2-EP)

4-ethylphenol; (4-EP)

### Supplier, City, State (Lot/Reference number)

EB: Sigma-Aldrich, St. Louis, MO (Lot No. 01353MC)

2-ethylphenol: Sigma-Aldrich, Milwaukee, WI (Lot No. 15418DO)

4-ethylphenol: Sigma-Aldrich, St. Louis, MO (Lot No. 08805HO)

### Purity/Characterization (Method of Analysis and Reference)

EB: 99.8% (analyzed by GC)

2-ethylphenol: 98.6% (analyzed by GC)

4-ethylphenol: 98.5% (analyzed by GC)

**CAS #**

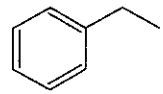
EB: 100-41-4

2-ethylphenol: 90-00-6

4-ethylphenol: 126-07-9

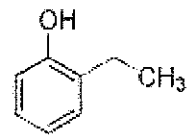
**Characteristics**

Chemical Structure

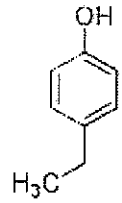


EB:

2-ethylphenol:




4-ethylphenol:




Molecular Formula

EB: $C_8H_{10}$

2-ethylphenol: $C_8H_{10}O$

4- ethylphenol: $C_8H_{10}O$

Molecular Weight

EB: 106.2

2-ethylphenol: 122.2

4- ethylphenol: 122.2

Boiling and melting points

EB: 136.2 °C; -95 °C

2-ethylphenol: 195-197 °C; -18 °C

4- ethylphenol: 218-219 °C; 40-42 °C

Density

EB: 0.8670 g/ml

2-ethylphenol: 1.037 g/ml

4- ethylphenol: 1.037 g/ml

Standards of Metabolites

(*S*)-(-)-1-phenylethanol (CAS 1445-91-6), 3,4-dihydroxyethylbenzene (4-ethylcatechol; CAS 1124-39-6), 2,4-dihydroxyethylbenzene (4-ethylresorcinol; CAS 2896-60-8), 2,5-dihydroxyethylbenzene (ethylhydroquinone, ethyl quinol; CAS 2349-70-4). Purity of each of the metabolites and name of the suppliers will be included in the study folder and in the final report.

## D. TISSUE TYPES AND SOURCE/SUPPLIER

This study will be conducted with microsomes prepared from liver and lung tissues obtained from rat, mouse, and human. Pooled liver and lung microsmes from male animals (F344 rats and B6C3F1 mice) and pooled human liver and lung microsomes (mixed gender) from non-smokers will be obtained from XenoTech (Lenexa, Kansas).

## E. BACKGROUND

Ethylbenzene (EB) is commonly used as an intermediate in the manufacture of styrene and synthetic rubber. It is also present in agricultural and home insecticide sprays, household degreasers, paints, adhesives, and rust preventives as a major component of mixed xylenes used as a solvent. Occupational exposure to EB may occur during the production of polystyrene as well as during production and use of mixed xylenes (Fishbein, 1985).

EB was not mutagenic in a variety of bacterial or yeast mutagenicity assays, either directly or in the presence of activating enzymes (Dean *et al.*, 1985; Nestmann *et al.*, 1980; NTP, 1999; Zeiger *et al.*, 1992). EB also did not increase sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells (NTP, 1999). In contrast, EB was reported to be weakly positive in a human lymphocyte sister chromatid exchange assay in the presence of activating enzymes (Norppa and Vainio, 1983) and to increase the incidence of mutations in a mouse lymphoma mutagenicity assay in the absence of activating enzymes. The latter response, however, was obtained only at cytotoxic concentrations in which growth was 13 –3 4% that of controls (MacGregor *et al.*, 1988) EB was negative in an *in vivo* mouse micronucleus assay (NTP, 1992, 1999).

No statistically significant increases in tumors were reported in Sprague-Dawley rats gavaged with 500 mg/kg/day EB (4 - 5 days/week, for 104 weeks) (Maltoni *et al.*, 1985) However, in an inhalation carcinogenicity study in which F344/N rats and B6C3F1 mice were exposed to 0, 75, 250, or 750 ppm EB 6 hours/day, 5 days/week, for 104 weeks, carcinogenic activity has been reported (NTP, 1999). Statistically identified neoplastic changes in the NTP (1999) study included: renal tubule adenomas in high exposure group male and female rats, lung alveolar/bronchiolar adenomas in high exposure group male mice (and intermediate exposure group when combined with carcinomas), and liver adenomas in high exposure group female mice.

The mechanism(s) of target-tissue specific tumorigenic activity of EB was evaluated by Stott *et al* (1999, 2001) by exposing rats and mice through inhalation of up to 750 ppm EB, 6 hours/day, for 5 or 28 consecutive days Exposure of EB caused an

increase in kidney weights in rats accompanied with an induction of CYP2B, CYP2E1 and UGT enzyme activities in male rats and inhibition of Phase I enzyme activities in female rats. Exposure to EB caused an increase in mouse liver weight and CYP1A and/or CYP2B enzyme activity in both sexes. In mouse lungs, CYP1A and CYP2B enzyme activities were inhibited in EB exposed animals.

Engstrom (1984) reported that the major metabolic pathways of EB in rats are its metabolism to 1-phenylethanol, 2-phenylethanol, 2-ethylphenol or 4-ethylphenol. Midorikawa *et al.* (2004) further reported that 2-ethylphenol and 4-ethylphenol are metabolically transformed to ring-dihydroxylated ethylhydroquinone and 4-ethylcatechol in phenobarbital-induced, Sprague Dawley rat liver microsomes at 5 mM concentration. Both of these dihydroxlated metabolites were shown to cause DNA damage, *in vitro*, in the presence of Cu(II). They concluded that these active dihydroxylated metabolites might be involved in the carcinogenesis induced by ethylbenzene.

Ethylphenols (2- and 4-ethylphenol) were found as minor metabolites of EB in the Phase I of the EB metabolism study (Saghir and Rick, 2005). The appearance of ethylphenols was above the detection limit (0.00053-0.0007 µmoles/ml incubation mixture) only in the mouse liver microsomes and rat and mouse lung microsomes and remained below the limit of detection in human liver and lung microsomes. The ethylphenols have been reported to cause direct pneumotoxicity in mice (Gelbke, personal communication, 2005). Since 2- and 4-ethylphenol have no structural alerts indicating cytotoxic potential, these findings point to the subsequent formation of ring-oxidized metabolites of ethylbenzene causing mouse specific lung toxicity. Although the Phase I study failed to identify significant formation of 2- and/or 4-ethylphenol by the mouse lung microsomes, Phase I studies were not designed to examine if any ethylphenols formed might have been rapidly metabolized to downstream catechol/hydroquinone, which may be the case for lung microsomes as reported for the liver microsomes by Midorikawa *et al* (2004).

Therefore, this study is designed to determine the potential of mouse, rat and human lung and liver microsomes to metabolize 2- and 4-ethylphenol to hydroquinone and catechol as well as rate of their formation from EB. Additionally, rate of the

formation of 1-phenylethanol from EB will also be determined between liver and lung microsomes among mouse, rat and human.

## F. STUDY DESIGN

### Part 1: Microsomal Metabolism of 2- and 4-Ethylphenol

One concentration of 2- or 4-ethylphenol (1 mM final concentration) will be incubated for 30 minutes with mouse, rat and human lung and liver microsomes (1 mg protein/ml 0.1 M phosphate buffer, pH 7.4, cofactors) (Table 1). The concentration was comparable to the extent of conversion of EB to these metabolites in Phase I studies and may provide enough metabolite(s) for analytical quanitifcation. After the completion of incubation, samples will be analyzed for the loss of 2- and 4-ethylphenol as well as formation of ethylhydroquinone and catechol either using Midorikawa et al. (2004) procedure or a technique using a trapping agent (e.g. adding excess GSH to the incubation mixtures) as was employed for the *in vitro* metabolism of 4-vinylphenol to hydroquinone and catechol (Bartels et al., 2004). Details employed will be included in the study folder and in the final report.

As a basis of comparison with the Phase I EB metabolism study (Saghir and Rick, 2005), mouse liver and/or lung microsomes will be incubated with 750 µg of EB delivered to 1mL of the incubation medium using DMSO as vehicle (or, 7 mM EB in test system). This is equivalent to the amount of EB delivered to the *in vitro* incubation systems of Phase I using 7500 ppm of EB vapor in the headspace and the highest amounts of 2- and 4-EP were generated by the mouse liver and lung microsomes in the Phase I study (Saghir and Rick, 2005). After completion of EB incubation, samples will be analyzed for the formation of 1-phenylethanol, 2-phenylethanol, acetophenone, 2-EP, 4-EP (as done in the Phase I study), as well as ethylhydroquinone and catechol

Table 1. Number of Incubations Per Substrate.

| | Tissue Type/Replicates | | |
|---|---|---|---|
| Species | Liver | Lung | Total[a] |
| Mouse | 3 | 3 | 6 |
| Rat | 3 | 3 | 6 |
| Human | 3 | 3 | 6 |

[a]Total = 39 or 42; 18 x 2 substrates (2-EP & 4-EP) + 3 or 6 EB incubations (mouse live rand/or lung).

**Part 2: Rate of Microsomal Metabolism of EB to 1-Phenylethanol, 2-Ethylphenol, 4-Ethylphenol, Ethylhydroquinone and Catechol**

Prior to the definitive incubations of EB with liver and lung microsomes of three species, to be described later, probe incubations of mouse liver and lung microsomes with EB will be conducted (at a single concentration) to assess the linearity of ethylhydroquinone and catechol formation over time (i.e., assess optimal incubation time to be used for the definitive kinetics experiments). Mouse liver and lung microsomes will be incubated with 7 mM EB (and 1 mg protein/ml 0.1 M phosphate buffer, pH 7.4, cofactors) in gas-tight vials. For each microsome type (liver and lung), incubations will be conducted for 10, 15, 30, 60, and 90 minutes (two replicate vessels/microsome type/incubation time).

Following the completion of the probe experiment to assess optimal incubation time, the definitive Part 2 experiment to determine kinetics of hydroquinone and catechol formation will be conducted. Liver and lung microsomes of three species (rat, mouse, human) will be incubated in triplicate with EB at six concentrations (Table 2). The substrate concentrations are tentatively proposed as 7.0, 3.5, 1.8, 0.9, 0.45, and 0.22 mM EB, but may be modified based on the results from the preliminary experiments. The incubation time will be selected based on the results of the probe experiments. Incubation solutions will be analyzed primarily for ethylhydroquinone and catechol using GC/FID, LC/MS, or GC/MS; but concentrations of 1-phenylethanol, 2-phenylethanol, acetophenone, 2-EP, and 4-EP will also be measured. Reaction mixtures may contain excess GSH to trap the reactive metabolites (i.e., quinones) which are formed during the incubations (and to determine their relative rates of formation between species and tissues). Details of the incubation conditions and analytical procedures will be included in the study file and final report.

Table 2. Number of Incubations/Substrate Concentration.

| | Tissue Type/Replicates | | | |
|---|---|---|---|---|
| Species | Liver | Lung | EB Conc. | Total[a] |
| Mouse | 3 | 3 | 6 | 36 |
| Rat | 3 | 3 | 6 | 36 |
| Human | 3 | 3 | 6 | 36 |

[a]Total samples : 108 samples.

## G. DATA ANALYSIS

Descriptive statistics (*i.e.*, mean ± standard deviation) for the depletion of parent EB and may be formation of metabolites will be calculated using Microsoft Excel® spreadsheets in full precision mode (15 digits of accuracy). If needed, statistical analysis will be performed to determine statistical significance among tissues and species. Rate of metabolism will be calculated using standard methods (e.g., Eadie-Hofstee, Lineweaver-Burk plots). Detail of the statistical methods employed will be included in the study file

## H. FINAL REPORT

A final report of the definitive study will be submitted to the study sponsor and will include but not be limited to the following:

1. Name and address of the facility performing the study and the dates on which the study is initiated and completed,
2. Objectives and procedures stated in the approved protocol, including any changes in the original protocol,
3. Statistical methods employed for analyzing the data,
4. Description of test and/or control substance (e.g., lot #, purity, physical characteristics, and method of preparing test concentrations),
5. Stability of the test and control articles under the conditions of administrations and description of the analytical methods used,
6. A description of the test system used including, when applicable, the number animals used, sex, body weight range, source of supply, species, strain, and substrain, age, and procedure used for identification,
7. A description of the dosage, dosage regimen, route of administration, and duration,
8. A description of all circumstances that may have affected the quality or integrity of the study,
9. Location of raw data and specimens,
10. List and signatures of study personnel,
11. Statement signed by the Quality Assurance Unit.

## I. REGULATORY COMPLIANCE

### GLP Standards

Japanese Ministry of International Trade and Industry (MITI)
GLP Standards Applied to Industrial Chemicals

US Environmental Protection Agency - TSCA GLPs
Title 40 CFR, Part 792 - Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards, Final Rule

Organisation for Economic Co-Operation and Development (OECD)
OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) EVN/MC/CHEM(98)17.

European Community
EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

### Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report will be inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

### Study Archives

The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

## J. SAFETY

Routine health and laboratory safety procedures will be followed when handling all test materials, radiotracer, animals and biological specimens. No other laboratory safety procedures are required

## K. REFERENCES

Bartels, M. J., Rick, D. L., Zhang, F. and Kan, L. (2004) In vitro metabolism of 4-vinylphenol and styrene in mouse, rat and/or human microsomes obtained from

lung and/or liver tissue. Report of The Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Dean, B. J., Brooks, T. M., Walker, G. H. and Hutson, D. H. (1985). Genetic toxicity testing of 41 industrial chemicals. Mut. Res. 153, 57-77.

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Maltoni, C., Conti, B., Cotti, G. and Belpoggi, F. (1985). Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. Am. J. Ind. Med. 7, 415-446.

Midorikawa, K., Uchidab, T., Okamotob, Y., Todab, C., Sakaib, Y., Uedab, K., Hirakua, Y., Murataa, M., Kawanishia, S. and Kojimab, N. (2004). Metabolic activation of carcinogenic ethylbenzene leads to oxidative DNA damage. Chemico-Biological Interactions 150:271–281.

National Toxicology Program (1992). Toxicity Studies of Ethylbenzene in F344/N Rats and B6C3F1 Mice (Inhalation Studies). Toxicity Study Report Series No. 10. NIH Publications No. 92-3129. U.S. DHHS, PHS, NIH, Research Triangle Park, North Carolina.

National Toxicology Program (1999). Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP TR 466, NIH Publications No. 96-3956.

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Norppa, H. and Vainio, H. (1983). Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. Mut. Res. 116, 379-387.

Saghir, S. A. and Rick, D. L. (2005). Ethylbenzene: in vitro metabolism in rat, mouse and human liver and lung microsomes. Report of The Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Stott, W. T., Johnson, K. A., Day, S. J. and McGuirk, R. J. (1999). Ethylbenzene: Mechanism of Tumorigenicity in Fischer 344 Rats and B6C3F1 Mice. Report of The Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Stott, W. T., Johnson, K. A., Day, S. J. and McGuirk, R.J. (2001). Ethylbenzene: Four-Week Mechanism of Tumorigenicity Study in Fischer 344 Rats and B6C3F1 Mice. Report of The Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals Environ. Mol. Mutagen. 19 (Suppl. 21), 2-141

Appendix B. Addenda to Protocol

# PROTOCOL CHANGE/REVISION

## TOXICOLOGY & ENVIRONMENTAL RESEARCH AND CONSULTING
## THE DOW CHEMICAL COMPANY, 1803 BUILDING, MIDLAND, MICHIGAN 48674

**Study Title:** ETHYLBENZENE: IN VITRO METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

**Study ID:** 051189

**CHEC File Number:** K-001271-027

**Change(s)/Revision(s) Number:** 1

**CHANGE(S)/REVISION(S):**

1) The rate of the formation of catechol and hydroquinone metabolites, and particularly reactive quinone metabolites derived from the formation of 2- and 4-ethylphenol (2-EP and 4-EP, respectively), was not monitored in the kinetics experiment. Those metabolites were monitored by GSH-trapping as described below

2) Monitoring the rate of metabolism of EB to quinone metabolite(s) (derived from 2-EP and 4-EP, which in turn were metabolized to catechols and hydroquinones) needed to be conducted using GSH trapping methods as these reactive metabolites may be rapidly consumed by reaction with incubation proteins. The Phase I metabolism study likely was unable to detect formation of quinone metabolites as a trapping system was not utilized in that study. In order to utilize the GSH-trapping technique, we needed to synthesize GSH conjugates of 2-EP and 4-EP-derived reactive metabolites (i.e., quinones) using radiolabeled ($^3$H-GSH) to produce an analytical standard to enable LC/MS/MS quantitation of the GSH conjugates of reactive metabolites (ultimately formed during the in-vitro incubation with EB). Synthesis of the $^3$H-GSH conjugates was quite complicated and required 120 hours (60 hours x 2 person) of time, which was beyond our anticipation at the time of writing protocol.

3) To conserve the mouse lung microsomes, we conducted the probe experiment, outlined in the Part 2 of the study protocol (to assess the linearity of formation of quinone metabolites of ethylhydroquinone and catechol over time), using only mouse liver microsomes. The concentration used was 2 mM not 7 mM as mentioned in the protocol.

4) As requested by Mike Gargas and Jim Bus, we included the determination of the rate of loss of EB in the study design which was not part of the original protocol. This addition increased the number of samples needed for incubation and especially the chemical analysis. The incubates needed to be extracted twice and run by GC/MS for parent compound and volatile metabolites (1-phenylethanol and acetophenone) and LC/MS/MS for the GSH adducts of the 2- and 4-EP derived ethylhydroquinone, quinone and catechol.

**REASON(S) FOR CHANGE(S)/REVISION(S):**

1) The low levels of 2- and 4-EP formation in Phase I of the study suggested the potential for rapid conversion of these metabolites to the more reactive ethylhydroquinone and catechol metabolites (and ultimately quinone metabolites). Thus, GSH trapping was necessary in order to accurately assess both the formation and rate of metabolism of EB to the final reactive metabolites.

2) See reasons above (#1). In order to properly monitor the rate of the formation of hydroquinone, quinone and catechol, $^{3}$H-GSH was necessary. This was needed for the synthesis of an analytical standard to facilitate quantitation of the GSH-trapped quinone metabolites by LC/MS/MS.

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix B. Addenda to Protocol (continued)



3) Methodology for measurement of quinone metabolite(s) formation was examined using liver microsomes in order to avoid method development work using expensive mouse lung microsomes This was to avoid depleting the lung microsomes we have in-house This not only saved money ($8350), but also avoided a potential delay in completing the study. If lung microsomes had been used in the probe experiments, as outlined in the original protocol, we would have exhausted all the leftover lung microsomes from our Phase I study (completed in 2005) and would have needed a new custom synthesis costing money and two months (see the attached quote outlining the cost and time). The concentration (7 mM) was well above the water solubility of EB; therefore, therefore the EB concentration used in the time course evaluation was reduced to 2 mM. In the final kinetics experiments (with liver and lung microsomes of the three species), incubations will be conducted with EB concentrations ranging from 0.22 to 7.0 mM.

4) This was added on the request by Mike Gargas. He needs this data in order to complete PBPK model. According to Jim Bus, the model can not be completed without having the rate of disappearance of EB

**IMPACT ON STUDY:**

1) No impact on study

2) Cost and time of completion

3) No impact on study.

4) Cost and time of completion.

**PLEASE FILE THIS CHANGE/REVISION WITH THE PROTOCOL**

D. L. Rick, B.S. — 9/28/06
STUDY DIRECTOR /DATE

S. A Saghir, M.Sc., M.S.P.H., Ph.D., DABT — 9/28/06
LEAD SCIENTIST /DATE

R. R. Albee, M.S. — 9/28/06
MANAGEMENT APPROVAL /DATE

**DISTRIBUTION** - See Attached List

ETHYLBENZENE: *IN VITRO* METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

Appendix B Addenda to Protocol (continued)

# PROTOCOL CHANGE/REVISION

**TOXICOLOGY & ENVIRONMENTAL RESEARCH AND CONSULTING**
**THE DOW CHEMICAL COMPANY, 1803 BUILDING, MIDLAND, MICHIGAN 48674**

**Study Title:** ETHYLBENZENE: IN VITRO METABOLISM WITH RAT, MOUSE AND HUMAN LIVER AND LUNG MICROSOMES – PHASE II STUDY

**Study ID:** 051189

**CHEC File Number:** K-001271-027

**Protocol Change(s)/Revision(s) Number:** 2

**CHANGE(S)/REVISION(S):**

1) The protocol states that EB will be added to incubation vessels using DMSO as the vehicle solvent. Instead propylene glycol was used as the vehicle to deliver EB to test vessels.

2) The protocol states that the incubations conducted to compare metabolite formation from incubations in which EB was introduced in a solvent vehicle (PG) against results from Phase I study (EB introduced as a vapor) would monitor formation of 1-phenylethanol, 2-phenylethanol, acetophenone, 2-EP and 4-EP as the bases of comparison. 2-phenylethanol and acetophenone were not analyzed in the probe comparative incubations.

**REASON(S) FOR CHANGE(S)/REVISION(S):**

1) Propylene glycol was judged by investigators to be a more suitable solvent than DMSO. Early incubations that compared metabolite formation using PG as the vehicle with incubations from the Phase I study (where EB was introduced as a vapor) produced very similar metabolite conversion results, confirming that PG was an appropriate delivery solvent.

2) It was decided to monitor for only 1-PE, 2-EP and 4-EP since 1-PE is the major metabolite formed in in-vitro incubations with EB and 2-EP and 4-EP formation were of a higher concern, at the early stage of the Phase II study, than 2-EP (which was only a minor metabolite in Phase I studies) and acetophenone.

**IMPACT ON STUDY:**

1-2) No impact on study

**PLEASE FILE THIS CHANGE/REVISION WITH THE PROTOCOL.**

D. L. Rick — 1/5/07
STUDY DIRECTOR /DATE

J. E. Battjes — 1/5/2007
MANAGEMENT APPROVAL /DATE

S. A. Saghir — 1/5/07
LEAD SCIENTIST /DATE