Report of the Peer Consultation Meeting on Ethylbenzene

Submission by
American Chemistry Council
Ethylbenzene Panel
for the
Voluntary Children's Chemical Evaluation Program (VCCEP)

February 22-23, 2007
Erlanger, Kentucky

Peer Consultation Organized by
Toxicology Excellence for Risk Assessment
(http://www.tera.org/peer/)

May 31, 2007

NOTE

This report was prepared by scientists of *TERA* and reviewed by the panel members. The members of the panel served as individuals on this panel, representing their own personal scientific opinions. They did not represent 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.

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Executive Summary

A panel of scientists with expertise in toxicology, exposure, risk assessment, physiologically-based pharmacokinetic (PBPK) modeling, and chemical mode of action (MOA) met on February 22 and 23, 2007, to conduct a peer consultation of a submission on ethylbenzene (CAS No. 100-41-4). The American Chemistry Council Ethylbenzene Panel and their contractors prepared the submission for the Voluntary Children's Chemical Evaluation Program (VCCEP). The purpose of the meeting was to provide a science-based forum to discuss whether the existing data are adequate to characterize the risks of ethylbenzene to children, and if not, to identify data needs. The sponsors and authors of the ethylbenzene submission provided the panel with summary presentations on the submission's assessments of hazard, exposure, risk characterization, and data needs. The panel then discussed the individual assessments presented in the submission, any additional data that were available, and the data needs.

The sponsor's exposure presentation identified two distinct sources of ethylbenzene: the ethylbenzene/styrene chain of commerce and the refinery chain of commerce. The presenter said the latter source provides over 98% of the ethylbenzene exposure to the general U.S. population, and ethylbenzene from this source always is combined with other petroleum chemicals. More than three-quarters of the refinery chain of commerce emissions originate from motor vehicles. The presenter described exposures to prospective parents and to children at specific life stages and presented information on the various types of exposure sources for each life stage. She also listed upper-bound and central tendency exposure data for the target populations and for several differing environments. Inhalation is the dominant ethylbenzene exposure pathway for all target populations. The presenter concluded that ethylbenzene production workers have by far the highest potential exposure to ethylbenzene. Among the general population, young children have higher exposures than older children or non-occupationally exposed adults, primarily due to the greater time spent indoors at home. Breastfeeding results in lower exposure than bottle feeding.

The panel considered a number of issues related to the exposure assessment. Individual panelists discussed potential exposures from localized sources such as groundwater contaminated from leaking underground gasoline tanks, daily use of ethylbenzene-containing products by subpopulations of non-production workers such as painters, and the use of ethylbenzene-containing consumer products by the general population. One panelist noted that the ethylbenzene indoor air concentrations used in the risk exposure assessment were mostly from long-term monitoring data; which may miss higher critical acute, episodic exposures of some target populations.

The sponsors provided two separate presentations on the hazard data assessment: one for the non-cancer toxicity studies and the other for the cancer studies. The first presenter emphasized that all of the hazard studies listed in the three tiers of the VCCEP pilot program had been conducted. She summarized each group of studies, noting that ethylbenzene was non-immunogenic and produced generally negative results in the genotoxicity assays. The chemical showed no adverse effects in reproduction studies or in the developmental neurotoxicity study; however, ethylbenzene ototoxicity was observed in a recent repeated inhalation exposure toxicity study conducted in rats. She concluded that ethylbenzene's non-cancer toxicity is well characterized, and she said the available data are adequate for human risk assessment. The second presenter summarized the cancer data from ethylbenzene bioassays in mice and rats. He

presented hypotheses for ethylbenzene's carcinogenic MOA in the kidneys of male and female rats, in the livers of female mice, and in the lungs of male mice. He noted that ethylbenzene is not genotoxic and emphasized that tumors occurred only at dose levels in the non-linear portion of the dose-response curve where normal metabolic pathways are saturated. He linked ethylbenzene's carcinogenic MOA to its metabolism in each of the organs where tumors occurred. He then described current and planned research to further explore the proposed MOA for the tumorigenic effects of ethylbenzene that were observed in the mouse lung. He concluded that the available information demonstrates ethylbenzene's carcinogenicity is unlikely to present a significant risk to human health at the current environmental and occupational exposure concentrations.

Several panel members discussed the neurotoxicity findings, with a few members expressing concern that the margin of exposure for ototoxicity might be decreased if there was concurrent exposure to ethylbenzene and other chemicals, such as toluene and the xylenes. Some panelists thought the submission did not adequately address all of the points listed in the U.S. EPA framework for evaluating cancer modes of action. In discussing the proposed modes of action for the rodent tumors, most panelists agreed with the sponsors that the rat kidney tumors and the mouse liver tumors were not relevant for human risk assessment. However, while agreeing with the sponsors that the MOA for mouse lung tumors was likely to be increased regenerative cell proliferation, some panelists thought this same MOA might occur in humans. They said obtaining more human metabolic data on ethylbenzene (as was proposed by the sponsors) would be critical in determining whether human pulmonary enzymes mimicked those in the mouse and could bioactivate ethylbenzene to produce lung tumors in humans.

The sponsors provided two separate presentations on risk characterization. The first presenter summarized PBPK modeling and the Reference Concentration (RfC) and Reference Dose (RfD) derivations. She described PBPK models in mice, rats, and humans, and explained how the sponsors derived toxicity reference values from the dose-response analysis of internal doses obtained from the PBPK models. She presented RfC and RfD values derived from oral and inhalation studies and explained the rationale used in considering different toxicity reference values and in determining the most appropriate values to use. The second presenter summarized the cancer reference value derivation. He said a threshold approach can be used for lung tumors, as well as for liver tumors, and he described this approach in detail. The presenter concluded that the available data are adequate for characterizing the risk to children and to prospective parents.

Several panelists discussed the ototoxicity, including its irreversibility and the appropriate uncertainty factors to apply to its point of departure. Members also discussed whether the mouse liver syncytial alterations might be a critical effect for the non-cancer endpoint. Many panel members voiced their agreement with the sponsors' cancer assessment methodology, presentation, and conclusions. They said ethylbenzene's lack of genotoxicity indicated a likely threshold, and cytotoxic metabolites within the lung likely caused proliferative regeneration resulting in lung tumors. Following extensive discussion of this topic, essentially all the panel members concluded that the rat kidney and the mouse liver tumors were not relevant for humans, and the mouse lung tumors were the critical effect most appropriate to use for human cancer risk assessment. As a result of this opinion from the panel, the sponsors stated that they will revise

certain sections of their submitted report so that the mouse lung tumor response is used as the cancer endpoint, while the rat lung metabolism data is used in the PBPK model and extrapolated to humans. Most panelists said the quantification of lung metabolism between mice and humans and between children and adults needs further exploration. One panelist recommended that the risk characterization also address non-production workers who are exposed to ethylbenzene at high doses on a daily basis.

In summarizing data needs, the sponsors stated that all of the toxicity studies listed in the three tiers of hazard tests for VCCEP had been conducted. They noted that the exposure, hazard, and risk characterization information contained in the submission had been supplemented by more recent data presented during the meeting. Taken together, they said the ethylbenzene data set was more than adequate to fulfill the requirements of VCCEP, and no data needs existed.

The panelists identified and discussed both data needs and data gaps. Because the authors already indicated they would revise their submission to respond to panel recommendations regarding MOA and selection of cancer endpoint, these items were not addressed by the panel members in the data needs discussion. Most panelists identified one or more data needs; however, many of these items were not related to ethylbenzene by itself, but rather to concurrent exposures to ethylbenzene and related chemicals, such as toluene and mixed xylenes. Panelists also identified data gaps, with the majority of the panel recommending that the report's description of ethylbenzene's carcinogenic MOA presentation be clarified and presented in a manner consistent with current U.S. EPA guidelines. In addition, single panelists identified a number of other data needs and gaps.

1. Participants

Sponsor

American Chemistry Council Ethylbenzene Panel (Chevron Phillips Chemical Company, The Dow Chemical Company, GE Plastics, INEOS Styrenics (formerly Innovene, formerly BP Amoco Chemical Company), Lyondell Chemical Company, NOVA Chemicals Inc, Sterling Chemicals, Inc., and TOTAL Petrochemicals USA, Inc. (Formerly ATOFINA Petrochemicals, Inc)

Presenters

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Lyondell Chemical Company

James Bus, Ph.D. Pharmacology, DABT
The Dow Chemical Company

Michael Gargas, Ph.D. BioMedical Sciences
The Sapphire Group

Janet Kester, Ph.D. Toxicology, DABT
NewFields LLC

Elizabeth Moran, Ph.D. Biochemistry, DABT
American Chemistry Council

Lisa Sweeney, Ph.D. Chemical Engineering, DABT
The Sapphire Group

Peer Consultation Panel Members[1]

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[1] Panel members served as individuals on this panel, representing their own personal scientific opinions. They did not represent 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.

Observers and Other Attendees

A list of observers and other attendees is found in Appendix A.

2. Background

This peer consultation meeting was organized by Toxicology Excellence for Risk Assessment (*TERA)*. *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. *TERA* has organized and conducted peer review and peer consultation meetings for private and public sponsors since 1996. Under this program, *TERA* is organizing peer consultation meetings for assessments developed as a part of the Voluntary Children's Chemical Evaluation Program (VCCEP). The ethylbenzene assessment was submitted by the American Chemistry Council Ethylbenzene Panel (Chevron Phillips Chemical Company, The Dow Chemical Company, GE Plastics, INEOS Styrenics (formerly Innovene, formerly BP Amoco Chemical Company), Lyondell Chemical Company, NOVA Chemicals Inc, Sterling Chemicals, Inc., and TOTAL Petrochemicals USA, Inc. (Formerly ATOFINA Petrochemicals, Inc).

The VCCEP program is a voluntary pilot program and part of the U.S. Environmental Protection Agency's (EPA) Chemical Right-to-Know Initiative (http://www.epa.gov/chemrtk/vccep/index.htm). The goal of the VCCEP is to enable the public to understand the potential health risk to children associated with certain chemical exposures. The key question of the program is whether the potential hazards, exposures, and risks to children have been adequately characterized, and, if not, what additional data are necessary. The EPA asked companies that manufacture and/or import 23 chemicals (that have been found in human tissues and the environment in various monitoring programs) to volunteer to sponsor chemical evaluations in a pilot program. 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 characterization assessment and a data needs assessment.

The VCCEP pilot program was designed to use a tiered testing approach. For toxicity data, specific types of studies have been assigned to one of three tiers. For exposure data, the depth of exposure information increases with each tier. Tier 1 hazard assessments use all available data, and therefore some of the Tier 1 chemical assessments will include toxicity studies indicated for Tiers 2 or 3. The Ethylbenzene Panel volunteered to sponsor a Tier 1 assessment for ethylbenzene. Links to the submission document and appendices are available to the public on the Internet at http://www.tera.org/peer/VCCEP/Ethylbenzene/EBWelcome.html. If data needs are identified through this process, the Ethylbenzene Panel will decide whether to volunteer for any additional data generation or testing and whether to provide a Tier 2 assessment for VCCEP peer consultation.

To provide wide-ranging scientific review of the sponsor's assessment, each submission undergoes review and discussion by a peer consultation panel in an open meeting with the public

invited to observe. The purpose of the meeting is to provide a science-based peer consultation on the data needs for the chemical, utilizing the assessment submitted by the sponsor, as well as the expertise and knowledge of the panel.

The VCCEP Peer Consultation Panel for the ethylbenzene submission consisted of 10 members independently selected by *TERA*. Each panel member disclosed information regarding potential conflicts of interest and biases for the VCCEP program in general, for ethylbenzene specifically, and for the ACC Ethylbenzene Panel (and its member companies and submission authors). *TERA* evaluated these disclosures in selecting the panel members. The disclosures were publicly presented at the beginning of the meeting (see Appendix B for the panelist disclosure statements). The panel members have experience in various disciplines, including toxicity testing, exposure evaluation, risk assessment, physiologically based pharmacokinetic (PBPK) modeling, and toxicology. The panel received a copy of the submission and key references approximately five weeks before the meeting, so that they had adequate time to review the documents and prepare for the discussions. Panel members bring a 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, rather the individual opinions of the members are noted. 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.

Members of the public were invited to observe the panel discussions by attending the peer consultation meeting or by viewing a live web cast of it. They were also given the opportunity to provide brief oral and written technical comments on the assessment document for the panel's consideration.

TERA prepared this meeting report. The report summarizes the sponsors' presentations, the panel discussions, the sponsors' comments during the discussions, and comments from the public. The meeting report is a summary, not a transcript. Individual opinions of the panel members are noted (although panelists are not identified by name), along with areas of agreement and disagreement. Panel members have reviewed the draft report and their comments, if any, have been incorporated in the final version. The sponsors also were given the opportunity to review the draft report to confirm the accuracy of their presentations and remarks. This report is available on the Internet at http://www.tera.org/peer/VCCEP/Ethylbenzene/EBWelcome.html.

This report is organized into sections corresponding to the submission: exposure assessment, hazard assessment, risk characterization, and data needs. Note that issues and concerns raised during the panel discussions did not always lead to recommendations for additional studies or data gathering.

3. Introductions, Conflict of Interest, and Meeting Process

The meeting opened with a welcome by Ms. Jacqueline Patterson of *TERA*. She described the background and purpose of the VCCEP peer consultation and the agenda for the meeting. Ms. Patterson noted that copies of panel members' biographical sketches and conflict of interest (COI) and bias disclosure statements were provided to all attendees (see Appendix B). The panel members then introduced themselves and noted whether they had additions or changes in their disclosure statements. No members had any substantive changes or additions.

Dr. Dourson, the panel chair, described how the meeting would be run. He explained that discussions would be based on the items found in the Charge to the Panel (located in Appendix B). He noted that all panelists would have the opportunity to state their own positions on the charge items, to ask one another clarifying questions, and to further discuss the issues. No attempt would be made to reach panel consensus positions on the charge items. The chair reminded the panel that the purpose of the peer consultation is not to review the adequacy of the submission document *per se*, but to determine the adequacy of the data for characterizing risk to children.

4. Sponsor Introduction

Dr. Elizabeth Moran of the American Chemistry Council (ACC) identified the companies on the ACC Ethylbenzene Panel that were sponsors of the ethylbenzene submission and outlined the presentations to be given during the meeting. She discussed the reasons that ethylbenzene had been selected for the VCCEP pilot program and reviewed the previous assessments conducted by various agencies on ethylbenzene. Dr. Moran described ethylbenzene's three sources: the refinery chain of commerce, the ethylbenzene/styrene chain of commerce, and a natural component of combustion of petroleum products. She noted that the sponsors of this VCCEP submission all were manufacturers in the ethylbenzene/styrene chain of commerce. See Appendix C for Dr. Moran's presentation slides, which provide further details.

5. Exposure Assessment

5.1 Sponsor Presentation

Dr. Janet Kester of NewFields, LLC, summarized the exposure assessment. She emphasized its objectives were to estimate chronic central tendency and upper-bound ethylbenzene exposures to the general public (prospective parents and children at specific life stages) as well as production workers, and also to determine the proportion of those exposures directly attributable to the ethylbenzene/styrene chain of commerce. She noted that the refinery chain of commerce was by far the greatest source of ethylbenzene exposure to the general U.S. population based on National Emissions Inventory data. Ethylbenzene exposure originating from this source always occurs with exposure to other petroleum-related chemicals, such as xylenes and toluene. She said the ethylbenzene/styrene chain of commerce contributed less than 2% of the total ethylbenzene exposure to the U.S. population. Dr. Kester presented information on the types of exposure

sources, such as ambient and indoor air, water, soils/sediments, food, household products, human milk, and polystyrene toys. She presented upper-bound and central tendency exposure estimates for the target populations and for several microenvironments. She concluded that production workers have by far the highest potential exposure to ethylbenzene. Among the general population, young children have higher exposures than older children or adults. Inhalation is the dominant exposure pathway for all target populations, with home air being the primary medium of exposure for all populations except production workers. Breastfeeding results in lower exposure than bottle-feeding. Further details of Dr. Kester's presentation are found in her presentation slides in Appendix C.

5.2 Clarifying Questions from the Panel

A panelist asked about the duration of the outdoor air sampling, but the authors did not have this information available. Panel members also asked about availability and use of EPA's Acute Exposure Guideline Levels (AEGLs) for comparison with acute exposure estimates. The authors noted that there are no AEGLs for ethylbenzene yet, but AEGL values for the mixed xylenes group of chemicals had been used for comparison in the ethylbenzene exposure assessment, even when the short-term exposures were considered to be non-emergency situations. The panelist thought that established limits for occupational exposures might have been better comparison benchmarks than AEGLs in some of the situations presented. Another panel member thought AEGL values in general were not appropriate to use for comparison benchmarks.

When asked about the lack of information presented on dermal exposure, Dr. Kester replied that dermal exposure to ethylbenzene was considered negligible. The panelist also asked for clarification of the exposure estimate for mouthing of toys (pages 6-54 and 6-55 of the submission). Dr. Kester explained that the authors assumed the entire inside surface of the mouth was exposed to the toy, although they realized this assumption would result in an overestimate of actual exposure.

5.3 Panel Discussion of the Exposure Assessment

The panel discussion of the exposure assessment addressed seven charge items, which are summarized in the sections that follow:

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

2. *Are the potential sources of ethylbenzene 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 are 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.*

5.3.1 Fate of ethylbenzene in the environment and human body

One panel member stated the exposure assessment had done an excellent job of environmental modeling, but another noted that ethylbenzene leakage into the ground from underground gasoline storage tanks at gasoline service stations had not been included. Dr. Kester acknowledged that such leakages exist, but she said that, in general, the level of ethylbenzene in groundwater is very low. Over 99% of ground water samples taken throughout the country were non-detect for ethylbenzene. The panelist maintained, however, that in those "hot spots" where leaks occur, the local population's exposure to ethylbenzene from groundwater might be very high.

Discussion of the fate of ethylbenzene within the human body was deferred until the hazard data presentation and discussions.

5.3.2 Sources of ethylbenzene

The panel discussed sources of ethylbenzene and whether the submission adequately addressed the important sources. One panelist thought the authors should have included more information on the ethylbenzene content of and exposure from household products. He suggested that a table listing key products might have been included in the report. Referring to page 6-16 in the submission, a panelist asked why the more recent data of ethylbenzene levels in homes, which showed lower levels than the previous data, were not used. The presenter said that the authors considered the earlier report (Shah and Singh, 1988) to be more reliable because it included a much larger number of records.

Considering sources of ethylbenzene in a larger context, a panel member distinguished "near" and "far" field exposures (see slide in Appendix D illustrating this concept). He noted that a person may be exposed to a substance from a "large, but distant environmental source (far)" and also from multiple "small sources within the microenvironment (near)". The panelist discussed the implications of this concept for ethylbenzene, and he emphasized that attention must also be

given to what he termed “near-field sources” of relatively short-term exposures occurring within a person’s microenvironment, such as household products.

A panelist noted that many people, who are not directly engaged in any of the chains of commerce identified by the authors, are exposed to ethylbenzene from working in jobs such as painting and automotive body repair. The panelist cited a paper by Jayjock and Levin (1984) describing exposures in small shops and noted that this population of workers is missing from the submission, and that children also might be exposed in many near-field operations occurring indoors.

5.3.3 Exposure data, scenarios, and subpopulations

The panel discussed the adequacy of the available exposure data; whether the appropriate data, exposure scenarios, age groupings and parameters were used; and whether the data were sufficient to characterize exposure for the key sub-populations. A panelist stated that the indoor air concentrations presented in the sponsor’s report represent primarily long-term (multi-day) monitoring or estimates from long-term outdoor ambient concentrations. These values might not include critical episodic exposure to ethylbenzene from the use of household products. The panel member noted that the sponsors’ report cites the work of Wallace et al. (1989) that painting and using a carburetor cleaner increased ethylbenzene exposure by 100-fold above background. The panelist expressed concern that periodic inhalation exposure to ethylbenzene 100-fold above background is likely to be extremely important in determining the relevant human exposure from this chemical. Because of the relatively short biological half-life of ethylbenzene in humans, the analyses of episodic exposures on the time frame of hours or a single day are critical to the evaluation of risk. Another panelist agreed that these concerns are valid when the health effects of interest are irreversible; however, the concern would not apply to long-term consequences of transient exposures if the body repaired itself.

Another panel member added that the kinds of effects observed from short-term exposures are best assessed by using the parameter of peak blood levels, rather than measuring the Area-Under-the Curve (AUC). This panelist also questioned whether the blood data in Table 6-2 were representative of the entire young adult population throughout the country, noting that the sampled subpopulations appeared to be non-representative of the country as a whole (i.e., the sampled groups were more than 50% rural; 43% low-income; 60% non-white.). The panelist thought the large sampling from the rural population might provide results of ethylbenzene exposure that were too low because ambient levels of ethylbenzene are lower in rural than in urban areas.

The panel discussed the age groupings. A panel member thought the age group of infants from birth to one year should have been subdivided further and was concerned that bottle-fed infants might be a key population not specifically addressed. The presenter clarified that the exposure assessment made the assumption that all infants from birth to one year were breast or bottle-fed for the entire year. When asked whether the *type* of infant formula might influence the amount of ethylbenzene exposure, the presenter replied that soy- or milk-based formulas both had similar ethylbenzene levels, so the formula type was not considered to be a meaningful exposure factor.

Another panelist observed that the exposure assessment concludes children 0-2 years old are exposed to higher levels of ethylbenzene via inhalation than are children aged 3-19, but noted that their P450 enzyme complement, particularly in the lung, which is a highly susceptible organ in neonates, is essentially unknown. The ability of respiratory tissues in children to detoxify reactive intermediates also is unknown. The panelist thought this lack of information on enzymes in this sub-population needs to be carefully considered when concluding whether further data are needed.

5.3.4 Exposure estimates and calculations

One panelist suggested that the PBPK model generated on a female rat could be modified to describe a specific state of pregnancy and be used to estimate the ethylbenzene exposure to the embryo and fetus.

The panel discussed the exposure calculations briefly. Panelists noted several general issues, including better explanations needed for table contents, the inconsistent use of mg/kg and ug/kg, and difficulty making comparisons because of the need to convert ppm to mg/kg/day. The following typographical error was noted: on page 6-54, the initial residual ethylbenzene concentration should be 29 ug/cm^3 instead of 27 ug/cm^3.

The authors agreed to revise the report to correct the errors and to better explain the sources and derivations of the values presented in the tables.

Addressing the assumptions made by the authors regarding oral exposure to ethylbenzene from the mouthing of toys, a panelist recommended the authors consider the total surface area of a toy that is mouthed during the exposure event or exposure duration (the area of the mouth in actual contact with the toy is not important in this approach).

5.3.5 Other issues related to ethylbenzene exposure

The panel discussed the conceptual exposure pathway model (Figure 6-7), and several members recommended additions to provide a more comprehensive picture of exposure. A panelist suggested the figure be expanded to show which pathways in the assessment were complete, which were incomplete (i.e., the pathway is possible in theory, but no actual human exposure is known to occur), and which had been quantified. Another panelist suggested the figure show (or at least reference) exposures occurring to painters, auto body repair workers, and others exposed to ethylbenzene in combination with xylenes and other petrochemicals. A third panelist suggested adding dermal exposure to this figure, even though the dermal exposure route was minor.

The issue of ethylbenzene being a component of mixed xylenes was discussed. The authors noted that the "Xylenes" chemical group was a previous submission under the VCCEP pilot program and was reviewed by a peer consultation panel on December 13-14, 2005 (see http://www.tera.org/peer/VCCEP/xylenes/xylenesWelcome.html for the sponsors' submission document and the peer consultation panel meeting summary). The VCCEP Xylenes submission included exposure evaluations and toxicity assessments of mixed xylenes, which includes

ethylbenzene. The authors noted that information regarding exposure levels and adverse effects is available in the xylenes submission and in the panel meeting summary. This information includes data on painting exposure scenarios and on acute toxicity. The presenters said mixed xylenes, which include 5-20 % ethylbenzene, purposely were not included in the ethylbenzene submission because the data are available in the xylenes VCCEP submission.

During a break in the meeting discussions, a panel member reviewed the VCCEP Xylenes submission. He reported back to the panel that modeling work had been done for human exposures from paints and degreasers that contain ethylbenzene, xylenes, and other chemicals. The information he reviewed compared the high-end 1-hour and 24-hour values to the AEGLs established for mixed xylenes. The information presented episodic exposures to painters that can be extrapolated to repeated exposures. Dermal exposures, as well as inhalation exposure, were considered. The panel member concluded that the estimated exposure to acute events with mixed xylenes was credibly presented in the VCCEP Xylenes report and the values could be used for ethylbenzene. The panelist did not believe, however, that using the same toxicological benchmark for ethylbenzene as was used for the xylenes (AEGL-1 = 130 ppm) was appropriate.

6. Hazard Assessment

6.1 Sponsor Presentations and Clarifying Questions

6.1.1 Non-cancer hazard data

Dr. Marcy Banton, Lyondell Chemical Company, summarized the non-cancer data available on ethylbenzene. She emphasized that the sponsors had completed the hazard studies listed in all three tiers of the VCCEP pilot program. Four of these studies were conducted by the Ethylbenzene Panel specifically for the VCCEP pilot program. Dr. Banton described each of the studies in the three tiers, discussing the health effect endpoint that the study evaluated and also the study results. She provided the LD50, NOAEL, and LOAEL values where appropriate. She noted that the studies found ethylbenzene was non-immunogenic and produced generally negative results in the genotoxicity assays. It showed no adverse effects in reproduction studies or in the developmental neurotoxicity study at the highest level tested (500 ppm). Ethylbenzene ototoxicity was observed in a recent repeated inhalation exposure toxicity study conducted in rats. Ethylbenzene has produced positive results in mouse and rat carcinogenicity studies. Dr. Banton concluded that ethylbenzene toxicity is well-characterized and the available data are adequate for human risk assessment. Animal toxicity tests showed adverse non-cancer effects at or above 200 ppm, while carcinogenicity was observed at 750 ppm with a NOAEL of 250 ppm. Dr. Banton noted that the general U.S. population exposure to ethylbenzene (1-2.5 ppb) is four to five orders of magnitude below the levels at which adverse effects are seen in animal studies. See Appendix C for Dr. Banton's presentation slides, which provide further details.

6.1.2 Clarifying question from the panel on non-cancer data

One panelist expressed concern that only one immunotoxicity study had been conducted. Dr. Banton replied this single study was sufficient because it gave no indication of any potential for

immunotoxic effects; in addition, results from the other animal studies gave no indication that the organs or tissues involved with immune response were affected by ethylbenzene exposure. The panelist responded that since autoimmune diseases often have involvement of both environmental and genetic factors, more meaningful studies should be conducted in rodent autoimmune models such as Brown Norway rats or MRL mice. Symptoms of autoimmune disease such as systemic lupus erythematosus (SLE) have been reported in populations exposed to water contaminated with chemicals such as benzene, toluene and xylene (Kilburn and Warshaw, 1992).

Another panel member asked about the functional observational battery (FOB) and motor activity results reported in the subchronic neurotoxicity test (Barnett, 2006). The panelist agreed with the submission's conclusions, but noted that the use of trend analyses on the within-session activity (linear trend in dose by linear or quadratic trend in time) is atypical and wondered why this particular approach was used. Dr. Banton referred the panel to pages 7-44 and 7-45 of the submission, which discuss this study. She said the approach used by Barnett was considered appropriate to address the dose-related trends that were observed.

A panelist wanted more information on *oral* dosing than was provided in the report. Dr. Banton replied that several oral studies had been conducted for Dr. Krishnan to use in his modeling work, and these studies could be made available. The panelist also noted that the survival rate of control animals in the NTP carcinogenicity study (NTP, 1999) did not appear to be adequate. Several panelists and sponsors reviewed the survival data from this study (summarized on page 7-38 of the report and presented in the Robust Summary on Appendix O). They determined that low survival did not occur in all the control groups but only in the male rats, and they concluded this effect was unlikely to affect the validity of the study.

6.1.3 Cancer hazard data

Dr. James Bus, Dow Chemical Company, discussed ethylbenzene's carcinogenic activity in rodent bioassays. He presented hypotheses for the chemical's carcinogenic MOA in the kidneys of male and female rats, in the livers of female mice, and in the lungs of male mice. See Appendix C for Dr. Bus's presentation slides, which provide further details. He noted that ethylbenzene does not show genotoxic potential and emphasized that the bioassays produced tumors only at the high dose level of 750 ppm, which is well into the non-linear portion of the dose-response curve where normal metabolic pathways are saturated. He stressed that ethylbenzene's modes of action for tumorigenicity were critically linked to its metabolism in all of the organs where tumors were produced. He proposed an exacerbation of chronic progressive nephropathy (CPN) as the MOA for the tumors occurring in rat kidneys and a phenobarbital-like enzyme induction effect as the MOA for the female mouse liver tumors. The MOA proposed for the carcinogenicity in male mouse lungs was increased regenerative cell proliferation caused by reactive metabolites produced within the Clara cells of the mouse pulmonary tissues. Dr. Bus described current and planned research to further explore the MOA proposed for the tumorigenic effects observed in the male mouse lung. To bring the panel up-to-date on the most recent findings of this work, he distributed copies of a study report completed February 20, 2007 from the Dow Chemical Company titled *Ethylbenzene: In Vitro Metabolism with Rat, Mouse and Human Liver and Lung Microsomes – Phase II Study* (a copy of this study is contained in

Appendix E). Dr. Bus concluded by stating that the available information demonstrates that ethylbenzene's tumor responses in rat kidney, mouse liver, and mouse lung are unlikely to present a significant risk to human health at current environmental and occupational exposure concentrations. He believes this conclusion will be further supported by additional research planned or in progress.

6.1.4 Clarifying questions from the panel on cancer hazard data

In response to several related questions from panel members, the presenter stated that metabolic viability of lung microsomes prepared from mice and rats could be more easily assayed than those from humans, while at the same time human lung metabolic viability was examined and confirmed present at reasonable levels relative to published values. He added that the methodology for preparation of rodent microsomes does not assure equivalent Clara cell content; however, it does capture total lung microsomal protein, most of which is contained with Clara cells. The presenter confirmed a panelist's supposition that ethylbenzene and the xylenes are metabolized differently within the mouse lung, and said this difference in metabolism likely is the reason that ethylbenzene is a mouse lung carcinogen, while the xylenes are not. He said a MOA involving oxygen radical formation within the ethylbenzene ring was not likely to occur because the monosubstitution of the ring favors other metabolic pathways. Responding to another question, the presenter said the MOA for the ototoxicity effect is not known, but it is believed to be caused by the parent compound.

Asked if genotoxicity studies were limited to rat liver preparations and if ruling out genotoxicity as a MOA was justified, the presenter replied that whole animal studies also had been conducted to assess potential genotoxicity and that proposing a non-genotoxic MOA for the rodent tumors was the most reasonable approach.

Referring to pages 7-39 and 7-40 of the submission, a panelist observed that there did not appear to be any threshold for the hyperproliferation response. Dr. Bus responded that enzyme induction and cytotoxicity both can be initiating events for cell proliferation. He said the high dose of 750 ppm resulted in both enzyme induction and in cytotoxicity, and tumorigenicity occurring only at the high dose indicates a cytotoxic threshold-related event in the mouse lung. Another panelist stated that the different responses in the lungs of mice and rats (e.g., tumors being produced only in the mice) were at least partially the result of higher P450 enzyme content in the lungs of the mice.

A panelist noted that quinones were listed as possible metabolites of ethylbenzene, and he wondered if the quinones might bind to proteins, thereby causing immunogenic activity. The presenter responded that protein adducts did not necessarily correlate with immunogenic activity.

6.1.5 Public Comment

Dr. Ines Pagan, a meeting observer from the EPA asked if ethylbenzene might be acting as a promoter and also if the bioassay on the ethylbenzene metabolite 1-phenylethanol (NTP, 1990) had produced any adverse effects other than kidney tumors. Dr. Bus answered that ethylbenzene

might possibly be a promoter. He said no adverse effects from the NTP mouse and rat bioassays on 1-phenylethanol had been reported except for increased tumors in male rats.

6.2 Panel Discussion of the Hazard Assessment

The panel discussion of the hazard assessment addressed three charge items, which are summarized in the sections that follow:

1. *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.*

2. *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.*

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

6.2.1 Adequacy of available ethylbenzene toxicity and ADME data

One panel member said the question of whether human lungs metabolized ethylbenzene had not been answered, and metabolic data from humans was needed to determine whether the human Clara cell CYP 2F1 enzyme mimicked the mouse Clara cell CYP 2F2 enzyme in ethylbenzene metabolic activity. The key question was whether the human lung bioactivates ethylbenzene. Another panelist agreed, adding that the Dow study distributed earlier in the day (Appendix E) provided important information on this issue, but further data were needed to compare the metabolic capabilities of mice and human lungs. Other panel members thought more information was needed to address the manner in which lung metabolism changes with age. The presenter responded that technology now was available to use "humanized mice" rather than doing experiments in humans or other primate species. One panelist explained that lungs of perinatal rodents are similar in development to human fetuses prior to birth, and rats do not have alveoli until post natal day (PND) four. These developmental differences will make it challenging to find the correct animal model for the infant human lung.

Several panel members discussed the adverse effect of ototoxicity and neurotoxicity. One panelist noted that 13 weeks of exposure in rats produced a NOAEL for ototoxicity of 200 ppm and a LOAEL of 400 ppm. The panelist thought that this toxic endpoint was important to include in the assessment, because of its small margin of exposure, and questioned why so little attention had been given to it in the report. Another panelist agreed with this concern, noting also that results of subchronic neurotoxicity studies showed effects where the NOAEL appeared to be 250 ppm for motor activity effects, rather than the 500 ppm NOAEL value that was reported. The ototoxicity effects lasted eight weeks, while the motor effects were more transient; therefore, the panelist considered ototoxicity to be the more important endpoint. This panelist also stated that excitability produced by central nervous system depressants are seen in humans, and these effects appeared to have been discounted in the sponsors' submission.

6.2.2 Data sufficiency for target subpopulations

Panel members raised several questions regarding hazard data for the subpopulations. A panelist wondered if the submission should address the children and neonate subpopulations directly by preparing PBPK models specifically for them. A presenter responded that PBPK models were traditionally constructed for adults and uncertainty factors (UFs) were used to extend the models to subpopulations such as neonates. She said it would be possible to construct a PBPK model specifically for neonates or other subpopulations. She reminded the panel that a Developmental Neurotoxicity (DNT) study had been conducted on ethylbenzene, but this study was not used as the basis for the ethylbenzene PBPK model that the sponsors were presenting.

A panelist thought ethylbenzene in human breast milk should have been discussed more, as it was found in human milk (page 6-5 of report). This panel member also expressed concern regarding a study showing increased mortality in runted animals (Stump, 2004) and asked that more information be obtained about the possible significance of this effect. Another panelist checked the historical control data from this strain of animal (Crl:CD(SD)IGS BR rats) in the evening after the first meeting day and reported the next morning that the increased mortality seen in this study was not far outside the normal range for the rat strain and was unlikely to have biological significance. This information and judgment satisfied the concerns of the first reviewer.

6.2.3 Cancer mode of action for ethylbenzene in laboratory animals and humans

A panel member stated that a credible evaluation of the human carcinogenic potential of a chemical requires that three questions listed in the *Framework for Evaluating Hypothesized Carcinogenic Mode of Action (Section 2.4.3 of the 2005 Guidelines for Cancer Risk Assessment, EPA 2005.)* be adequately addressed. If there is evidence for more than one MOA, each MOA should receive a separate analysis. These questions are:

1. Is the hypothesized MOA sufficiently supported in the test animals?
2. Is the hypothesized MOA relevant to humans?
3. Which populations or life stages can be particularly susceptible to the hypothesized MOA?

Panel members discussed whether these questions had been adequately addressed for each of the three situations in which animal tumors had been produced: in rat kidneys, in female mouse livers, and in male mouse lungs. Several panelists voiced their agreement with the rationale presented in the report and with the authors' conclusion that the carcinogenic effect produced in rat kidneys was not relevant for humans. However, one stated that the report should provide additional explanation to make it more obvious why CPN will not occur in humans. A panel member thought a stronger correlation should be made between a-2u-globulin and cancer, but anther member said this correlation already was well documented in the publications of Hard (2002) and of Stott (2003). None of the panel members expressed disagreement with the proposed MOA for tumor production in the rat kidneys or with the authors' conclusion that this effect seen in rats was not relevant for humans.

Regarding the tumor production in the mouse liver, a panelist thought the submission should further support the proposal that the MOA was similar to phenobarbital-like enzyme induction, but agreed with the sponsors that the mouse liver tumors were not relevant to humans. When asked if there was any evidence at all for reactive metabolites in the mouse liver, Dr. Bus responded that a small amount of ring oxidation was known to occur, but the enzyme induction response was the main occurrence and was the mechanism believed to be the cause of the cancer. Another panelist said many humans take phenobarbital over their lifetimes and have shown no indication of increased incidence of liver tumors. None of the panel members expressed disagreement with the MOA proposed for tumor production in the mouse liver or with the authors' conclusion that this effect in mice was not relevant for humans.

In addressing the MOA proposed for the tumors produced in the mouse lungs, the panel discussed the February 2007 report from the Dow Chemical Company (Appendix E), which was distributed at the meeting. One panel member voiced agreement with the sponsors that the MOA for the mouse lung tumors was increased regenerative cell proliferation; but thought this same MOA might also occur with ethylbenzene in humans. The panelist believed the study results that were presented addressed differences between mice and rats, but not between mice and humans. This panelist said discounting the mechanism of increased regenerative cell proliferation as a possible MOA for ethylbenzene in humans was premature. Another panelist said the results of the planned CYP 2F2 knock-out/CYP knock-in animal experiments would be a key factor in determining if the effects observed in the mouse lung might occur in humans. The panelist cautioned that it would be difficult to assure that experiments comparing the enzyme activities of lung tissues from mice and humans contained equivalent percentages of Clara cells, and noted that Clara cells from mice and rats contained vastly different amounts of 2F2 and 2F4 enzyme activity. Another member added that human lungs have a lower percentage of Clara cells than do the lungs of mice or rats, but the alveolar and other epithelial cells in human lungs might be able to metabolize ethylbenzene in the same way that rodent Clara cells do. Several panelists discussed the enzymatic metabolism data presented in Table 1 (page 41) in the Dow report and asked the presenter if the relatively low level of activity shown for the human lung might indicate a loss of viability. Dr. Bus responded that viability tests had been conducted on the commercially obtained human tissues, and the results confirmed the tissues were viable. Some of panel members continued to express concern about the lower-than-expected human lung microsomal activity, and they suggested lung microsomal metabolism data from *other* species should be considered for extrapolation to humans.

6.2.4 Other issues related to the ethylbenzene hazard assessment

One panelist remarked that the human data are sparse and mostly consist of effects observed following exposures to ethylbenzene-containing mixtures, rather than to ethylbenzene alone. A presenter added that human occupational exposure to ethylbenzene is generally about 1 ppm, while the AEGL value for xylene mixtures that contain ethylbenzene is 130 ppm.

Speaking more generally about all the chemicals in the VCCEP pilot program, a member observed that other VCCEP panels have expressed concern regarding the relative lack of metabolic data existing for young animals and for children, and said this is an important need that should be addressed. Another panelist responded that although having such metabolic

information would be helpful, it would not account for the differing types and amounts of *exposures* that occur at the various life stages.

7. Risk Characterization

7.1 Sponsor Presentations and Clarifying Questions

7.1.1 PBPK modeling and RfC and RfD derivations

Dr. Lisa Sweeney of the Sapphire Group presented the section of the risk characterization assessment dealing with PBPK modeling and the RfC and RfD derivations (see Appendix C for her presentation slides, which include further details). She described the PBPK models that have been developed for ethylbenzene in mice, rats, and humans and explained how each of these models was modified and used in the sponsors' submission. The human model was modified to estimate lactational transfer from mothers to infants and to enable upper-bound estimates of reactive metabolites formed in the human lung. Dr. Sweeney explained that the toxicity reference values were derived via dose-response analysis using benchmark dose software based on internal doses obtained from PBPK models. Uncertainty factors were applied to the points of departure, and the human-equivalent internal doses were transformed to external toxicity reference values using the PBPK model for route-to-route and interspecies extrapolation. She presented RfC values that had been derived for several toxicity endpoints. She also presented RfD values that had been derived from oral and from inhalation studies. Dr. Sweeney explained the rationale the authors used in considering the different toxicity reference values that might be used. She concluded that the most appropriate RfC value to use for the ethylbenzene risk assessment was 0.8 ppm and the most appropriate RfD to use was 0.5 mg/kg-day. Both of these toxicity reference values were based upon a chronic inhalation study in mice (NTP 1999).

7.1.2 Clarifying questions from the panel

A panel member asked what strains of rats had been used in the oral and inhalation studies conducted on ethylbenzene. Dr. Sweeney responded that Wistar, Sprague-Dawley, or Fischer rats had been used in the studies. Details on the strains of each animal species used in each study are available in the robust summaries of the toxicity studies, which are contained in Appendix O of the submission.

Other members expressed concern that the adverse effect of ototoxicity had not been given sufficient consideration in determining the toxicity reference doses. They discussed the difficulty of determining when cochlear hair loss really occurred and if the hair loss itself was a critical effect or an immediate precursor to the critical effect of hearing loss. Dr. Sweeney replied that to be conservative, they considered just 1% cochlear hair loss to be an adverse effect, and she referred the panel to the discussion of the proposed MOA for ototoxicity and its internal dose metric on page 8-5 of the submission.

A panelist note, and the presenter agreed, that on page 8-15 (Section 8.2.3.5) in the calculation of the default RfD, the calculation should be "Default RfD = 75/1000 = 0.08 mg/kg bwt/day" and the duration adjustment of (36/168) should be omitted.

7.1.3 Cancer reference value derivation

Dr. Michael Gargas of the Sapphire Group presented the section of the risk characterization assessment dealing with the cancer reference value derivation (see Appendix C for his presentation slides, which include further details). He said the authors believe the modes of action displayed by ethylbenzene for cancer in animals are not likely to be relevant to humans, and they used a threshold, "RfC-type" approach for the cancer value derivation. He said this approach can be used for *lung* tumors as well as for *liver* tumors. He noted that Appendix T of the submission used mouse *liver* effects as a basis of the cancer reference value, but this is now not recommended because of the panel's conclusion during the first day of the meeting that mouse liver cancer effects are not relevant to humans (see Section 6.2.3 above for the panel discussion). Dr. Gargas explained that because the animal tumors of interest for human risk assessment are now the *lung* tumors, rather than the *liver* tumors, the reference values for cancer that are presented in the submission and slides are no longer correct. The total hazard indices for the most highly exposed child and most highly exposed prospective parents must be divided by a factor of 1.6 to be correct. *[NOTE: The sponsors intend to issue a revised submission report that incorporates these corrections.]* Dr. Gargas stated that these changes do not affect the conclusions of the risk characterization assessment. He concluded his presentation by stating the available ethylbenzene data are adequate for characterizing the risk to children and to prospective parents. Human exposures are extremely low, while animal studies demonstrate that toxicity does not occur below air concentrations of 200 ppm (non-cancer effects) or 750 ppm (cancer effects).

7.1.4 Clarifying questions from the panel

One panelist observed that the authors had applied their UFs to *internal* doses rather than external doses and noted that this is not standard practice. The presenter replied that the standard practices are intended to allow flexibility. The panelist agreed, but thought that the report should have provided a rationale for deviating from the norm. The panelist added that the kinetics of the internal doses must be linear to apply the traditional UFs to them, but acknowledged that the issue of using traditional UFs for internal doses was an area of active discussion within the scientific community.

7.2 Panel Discussion of Risk Characterization

The panel discussion on Risk Characterization addressed six charge items:

1. *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.*

2. *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.*

3. *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.*

4. *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).*

5. *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.*

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

7.2.1 Noncancer toxicity benchmark values proposed for ethylbenzene

Regarding ototoxicity, panel members questioned the use of a UF of 1 for a subchronic-chronic extrapolation. One noted that the basis of this decision was a study on toluene, not on ethylbenzene and added that although chronic studies on both ethylbenzene and styrene exist, ototoxicity was not evaluated in those studies. Another member asked about whether the combined effect of ethylbenzene exposure and concurrent noise had been evaluated. A presenter responded that acute studies showed noise had no effect on the threshold for ethylbenzene ototoxicity. A panelist said that because the cochlear hair loss from ethylbenzene was irreversible, over time it could combine with the cochlear hair loss that occurs naturally with aging to cause hearing loss sooner than would otherwise be the case. Therefore, the UF for chronic-to-subchronic extrapolation should be greater than 1, and a UF of 3 seems more appropriate. Another member thought using 1% hair loss was highly conservative and favored keeping the UF at 1. Other panel members expanded the discussion to include whether peak blood levels or AUC should be used as the proper assessment parameter, whether cochlear hair

loss or functional hearing loss should be used as the critical effect, and whether using the critical effect or a direct precursor of the critical effect would be best to use in this case. The panelists who participated in this discussion appeared evenly split over the question of whether a value of 1 or 3 should be used for the subchronic-to-chronic UF.

One panelist commented on the liver syncytial alteration observed in male mice (page 8-6 of the submission) and wondered if it might be a critical effect for the non-cancer endpoint. Another panelist responded that a few cells in adult rodent livers have two nuclei, but, in this case, the hepatic effects described (syncytial alteration) appeared to be qualitatively different and might be related to central lobular necrosis. A third panel member noted that the syncytial alteration did not seem to be correlated with any functional impairment, and suggested it might be a functional precursor. No one else commented on this issue, and the possibility of the mouse hepatic syncytial alteration being a critical non-cancer effect remained unresolved.

7.2.2 Modes of action for ethylbenzene carcinogenicity

As noted above in Section 7.1.3, after considering the panel's previous discussions, which concluded (1) that the rat kidney and the mouse liver tumors are not relevant for humans, and (2) that the mouse lung tumors are the critical effect most appropriate to use for human cancer risk assessment, Dr. Gargas and the sponsors stated that they would revise their written submission to incorporate the comments the panel had provided earlier in the meeting. Specifically, the revised submission will use the mouse lung tumor response for the cancer endpoint, while rat lung metabolism data will be used for the PBPK model and for extrapolation to humans. One of the panelists remarked that extrapolating rat metabolism instead of mouse metabolism to humans might be too conservative, even though the rat-to-human extrapolation has been more studied and is likely to be more accurate. When asked if PBPK data from other chemicals could be used to compare metabolism in the mouse and rat, Dr. Sweeney responded that ethylbenzene metabolism in the mouse is higher than in the rat as demonstrated in the new Dow study provided by Dr. Bus, so there is no need to compare metabolism of other chemicals in mouse versus rat lungs. A panelist added that the MOA for ethylbenzene carcinogenicity in mouse lung is plausible and might be relevant for humans. The current work by Dr. Bus should further clarify these issues and might show that the MOA in the mouse is not plausible in humans.

A meeting observer, Dr. George Cruzan of ToxWorks, added that the enzyme CYP 2F2 is needed for styrene toxicity, and CYP 2E1 probably does not contribute to styrene toxicity in mice. A panelist replied that many compounds are bioactivated to toxic moieties via P450 enzymes. CYP 2E1 might be different in mice and humans. Mouse lung is usually more active than human lung, and it is not predictive of response in other species. Because the relative distribution of these key metabolic enzymes in the lungs of mice and humans is not known, it appears justified to use the entire lung as the source of these enzymes, even though the lung is know to be comprised of many different cell types. Inactivation of pulmonary enzymes also may occur as a result of chemical exposures ("suicide inhibitors"), as is discussed in the Dow report (Appendix E).

7.2.3 Validity of the ethylbenzene cancer dose-response assessment

Several panel members expressed agreement with the cancer assessment methodology, presentation, and conclusions, and one referred the panel specifically to Figure 8-5 on page 8-43 of the report as support. The panelist thought that ethylbenzene's lack of genotoxicity indicates a likely threshold, and cytotoxic metabolites within the lung cause proliferative regeneration resulting in lung tumors. If ethylbenzene's pulmonary cytotoxicity has a threshold, then ethylbenzene's pulmonary carcinogenicity also has a threshold. The panelist identified ethylbenzene's quinone metabolites as possible mutagens that might be directly responsible for the lung tumors, but acknowledged that no direct evidence existed to support this as the real carcinogenic mechanism. Another panelist said that the report's presentation of its cancer assessment rationale might be simplified to make it more understandable for non-cancer specialists.

7.2.4 Differences in ethylbenzene metabolism among species

All the panel members agreed that PBPK data contained in the report supported the existence of biologically meaningful differences in ethylbenzene metabolism among the species investigated (mouse, rat, and human), but most said the quantification of lung metabolism between mice and humans needs further exploration. One member reminded the panel that a key aspect of the difference in metabolism was the relatively low activity of the microsomal enzymes demonstrated by the commercially obtained human lung tissue and emphasized the importance of assuring maximum viability of the human tissues. Another member added that it was not clear that Clara cells had been isolated from the mouse or human cultures. If they were not, and if the density of Clara cells at the locations sampled in the two species differed, then the species comparisons presented would not be accurate.

7.2.5 Risk characterization to target subpopulations

To facilitate discussion of this question, the panel chair referred the panel to the tables on pages 9-6 and 9-7 of the sponsors' report. *[NOTE: As mentioned above, Tables 9-1 and 9-2 on pages 9-6 and 9-7, together with the text accompanying these tables, will be revised by the authors. The sponsors will issue a revised submission report that incorporates these changes. The paragraphs below summarizing the panel discussion of this Charge Item assume that the revisions described above will have been made, and, as a result, all the values listed in Tables 9-1 and 9-2 will have been approximately doubled.]*

One panelist, referencing the publication of Jayjock and Levin (1984), recommended that an additional column be added to Tables 9-1 and 9-2 to provide information on the subpopulation of non-production workers (e.g., painters, auto body workers, etc.) who are exposed to ethylbenzene at high doses on a daily basis. The panelist also suggested that Table 9-1 be expanded to present the chronic and acute (one-day) exposures separately, as was done in the VCCEP Xylenes submission. A presenter noted that the requested additional columns and data would reflect exposures to the mixed xylenes group of petrochemicals that contained ethylbenzene, rather than to ethylbenzene alone; therefore, the reference benchmark values from

the VCCEP Xylenes submission would be more appropriate to use. The panelist disagreed with the use of the Xylenes reference benchmark values, saying the VCCEP Xylenes submission did not include the hazard endpoint of ototoxicity; therefore, a new reference benchmark value would need to be derived. The panelist noted that the AEGL-1 value for mixed xylenes is 130 ppm, while the reference benchmark value for ethylbenzene ototoxicity is 1 ppm. The presenter pointed out that ototoxicity *was* included in the AEGL-1 value for mixed xylenes, as well as for the AEGL values now being developed for ethylbenzene.

The panel further discussed the ototoxicity endpoint and the ethylbenzene studies. One panelist noted that the rodent two-generation and DNT studies conducted on ethylbenzene could not provide data on ototoxicity for embryos, fetuses, neonates, or young children. Another pointed out that the Organ of Corti is not fully developed in the rodent embryo/fetus and matures after birth. Furthermore, the functional observational battery (FOB) of the DNT study is not designed to detect ototoxicity.

Several panel members thought lung metabolism in children is likely to be equal to or less than the lung metabolism in adults, but they said this issue needs further exploration. Other panelists mentioned that it would have been informative if the ontogeny of key enzymes (especially CYP 2F1) had been presented in the submission, perhaps as a separate model for the embryo/fetus and the neonate.

7.2.6 Public comment

Dr. Ines Pagan, an observer in the audience from EPA, recommended that the sponsors use a weight of evidence approach to evaluate what she termed the "key events" of enzyme induction, cell hypertrophy, and cell apoptosis. She believed it was not correct to conclude that tumors noted in the rodent studies are never relevant to humans.

8. Data Needs

8.1 Sponsor Presentation

Dr. Elizabeth Moran of the ACC briefly summarized the information that had been presented earlier in the meeting. She emphasized that every EPA-recommended toxicity test in all three of the tiers had been conducted, plus additional studies had been performed to explore ethylbenzene's MOAs. She noted that ethylbenzene had been assessed and was regulated by numerous governmental agencies, human exposures to the chemical were known to be low, and there was no evidence of increased susceptibility in any target subpopulation. For these reasons, Dr. Moran concluded that there were no hazard or exposure data needs for ethylbenzene. She said the absence of data needs was especially true in the context of what the expectations are for the VCCEP pilot program.

8.2 Clarifying Questions from the Panel

In response to a question from a panelist of why the sponsors were funding additional work (described previously in the meeting by Dr. Bus) if they believed no data needs existed, the presenter said the additional work was part of a larger product stewardship effort to further understand ethylbenzene's MOAs in different tissues and species. She said these current and future experiments were outside the scope of the data needs intended for VCCEP.

8.3 Panel Discussion of Data Needs and Gaps

The panel discussion on the Data Needs Assessment addressed two charge items:

1. 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.

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

The individual panelists discussed items that they considered to be data gaps and data needs. The panel chair explained that in the context of the VCCEP pilot program, *data gaps* are defined as areas that could benefit from additional data, additional studies, additional analysis, or clearer presentation. *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, and the panelists may consider the risk characterization results when determining whether a *data gap* is a *data need*

8.3.1 Data Needs

Two panel members did not identify any data needs. The other eight panel members identified items that they considered to be needs for the hazard assessment, exposure assessment, or risk characterization. Many of these items were not related to ethylbenzene by itself, but rather to concurrent exposures to ethylbenzene and related chemicals, such as toluene and mixed xylenes. Because the authors indicated that they would be making revisions to the submission document based on the panel's comments earlier in the meeting (i.e., using the mouse lung tumor response as the cancer endpoint and using the rat lung metabolism data in the PBPK model extrapolated to humans), the panel members did not address those specific changes in their identification of data needs.

A better understanding of the ototoxicity endpoint resulting from exposure to ethylbenzene alone and in combination with similar chemicals was a concern to a number of panel members. Two panelists thought a UF of 3 was warranted for subchronic-to-chronic extrapolation of the ototoxicity effect. One of the panelists suggested that a UF of less than 3 might be used if chronic studies of ototoxicity were conducted or other mechanistic studies done to address chronic exposure and provide more information to reduce the uncertainty factor. Another

thought that, in general, further information is needed regarding the relationship of cochlear hair loss to hearing loss. Other panelists identified this item as a data *gap*, not a *need*. They said additional discussion of this topic would be helpful, as well as consideration of the sensitivity of ethylbenzene's ototoxicity during *in utero* and neonatal life stages. One panelist pointed out that ethylbenzene's ototoxicity has been much less studied than the chemical's cancer endpoints, even though it appears to be a critical effect at levels below metabolic saturation. This panelist identified the need for well-considered exposure limits for workers with repeated exposures (i.e., 40 hours a week, 50 weeks a year). This panel member also identified the need for an episodic exposure limit based on ototoxicity for children using a one-day averaging time for inhalation exposure. The panel member did not support the use of AEGLs for these exposure scenarios because AEGLs, as they are conceptually defined in this panel member's opinion, are not appropriate to apply to reasonably anticipate episodic exposures of children to household products.

In regard to the sponsors and authors statement that they planned to revise the submission to include discussion and findings from the recent Dow report (Appendix E) presented during the meeting, several panelists identified the need for more hypothesis-driven experiments to investigate the metabolism of ethylbenzene in the human lung. Specifically, one member identified as a need the calibration of ethylbenzene metabolism in the human lung, using the Dow report data and then re-running the PBPK model. Another panel member thought that exploration of the susceptibility of human pulmonary cell lines to cytotoxicity from ethylbenzene and quinone was a data need. A third panel member noted that the ongoing studies might place too much emphasis on P450 and the investigators should also pay attention to the Phase II enzymes and products.

Another panel member identified as a need the conducting of studies to measure the kinetics of recombinant enzymes to better understand human metabolism and time-dependent activation. Another identified the need for more understanding of the role of the Clara cells.

Two panelists identified a data need for the exposure assessment. They thought the submission should evaluate and quantify exposure for several additional scenarios that involved concurrent exposures to ethylbenzene and similar chemicals. These scenarios include child hobbyists using ethylbenzene-containing products, other child and adult household product exposures (similar to what has been done in previous VCCEP assessments), and adult non-production workers. The panelists defined non-production workers as those typically involved in small-scale industrial or commercial concerns, which have little or no governmental or other occupational health services available (e.g., small automotive body repair shops or painters). They suggested that the VCCEP mixed xylenes assessment might provide much of the needed information, but they recommended that this information be discussed explicitly in the ethylbenzene submission, rather than just cross-referenced to the VCCEP Xylenes submission.

8.3.2 Data Gaps

Panel members identified what they considered to be data gaps – those studies or analyses that would be *helpful* to better understand ethylbenzene exposure, hazard and risk, but were not *necessary* to characterize risks to children. Many of these gaps address further explanations and

discussions that would be useful to include in a revised document, while others address additional scientific studies, which may advance general scientific, as well as ethylbenzene-specific, understanding.

Most of the panel members thought that the carcinogenic MOA presentation in the report should be clarified and presented in a manner consistent with current guidance such as EPA's *Framework for Evaluating Hypothesized Carcinogenic Mode of Action* (EPA, 2005). They identified this as a data gap because they did not believe new work was needed, but the presentation in the report should be clarified. They said the report should provide a clear step-by-step presentation of the authors' rational in determining the human relevance of the proposed modes of action. One panel member noted specifically that the chronic progressive nephropathy MOA needs to be described better and its non-relevance to humans more fully justified. The authors should go beyond simply citing the Hard (2002) paper and incorporate a step-by-step explanation.

Several panelists identified the ontogeny of pulmonary and hepatic enzymes in laboratory animals and in humans as a data gap. One suggested using the PBPK model to simulate metabolic capabilities at different life stages. Another suggested more data and information on lung metabolism, specifically on the CYP 2F1 (human) and CYP 2F2 (animal) enzymes.

Single panel members identified a number of additional data gaps. In the exposure area, data gaps included revising the toy-mouthing scenario to make it more realistic and evaluating the exposure of local subpopulations to ethylbenzene from ground water contaminated with petrochemicals.

Other items identified as data gaps by single panelists were: exploring effects of early-life exposures on diseases or adverse effects occurring in later life; further discussing the possible human relevance of the FOB effects seen in the acute and subchronic studies; conducting additional immunotoxicity testing in immunodeficient animals; exploring whether ethylbenzene behaves like phenobarbital in the mouse liver; explaining and justifying the use of UFs for the internal doses obtained from the PBPK modeling; considering dropping the high dose BMD values used in some calculations; and performing experiments on the biochemical process of neoplasms to help determine if CAR (Constitutive Androstane Receptor) mediated induction is occurring.

9. Addendum

After the meeting, a panelist notified *TERA* that the panelist had intended, but failed, to raise for panel discussion a recommendation to conduct a mode of action/human relevance analysis for the male rat Leydig cell tumors observed in the NTP bioassay (NTP, 1999). *TERA* advised the submission's sponsors of the panel member's post-meeting comment, and the sponsors indicated that they intend to address this tumor type in their revised submission.

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