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Scoping and Problem Formulation Materials
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Scoping and Problem Formulation for the Identification of Potential Health Hazards for the Integrated Risk Information System (IRIS) Toxicological Review of Ethylbenzene

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NOTICE

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CONTENTS

PREFACE

The National Research Council's Review of EPA's Integrated Risk Information System (IRIS) Process (NRC, 2014) discussed scoping and problem formulation as they apply specifically to IRIS assessments. IRIS assessments evaluate the available scientific literature to identify potential human health hazards of a chemical and to characterize dose-response relationships for each hazard. Accordingly, the NRC discussed scoping and problem formulation for IRIS assessments as being restricted to scientific questions that pertain only to hazard identification and dose-response assessment. Exposure assessment and risk characterization (the other components of a risk assessment) are outside the scope of IRIS assessments, as are the legal, political, social, economic, and technical aspects of risk management.

During scoping, the IRIS program seeks input from EPA's program and regional offices to identify the information and level of detail needed to inform their decisions. This includes the exposure pathways and specific exposed groups that the assessment will consider. The NRC's Review of EPA's IRIS Process characterized this practice as consistent with the risk-assessment guidance in Science and Decisions (NRC, 2009).

During problem formulation, the IRIS program seeks input from the scientific community and the general public as it frames the specific scientific questions for the systematic reviews that it will conduct in the assessment. The NRC's Review of EPA's IRIS Process identified the major challenge of problem formulation as determining which adverse outcomes the assessment should evaluate. The NRC suggested a three-step process for conducting problem formulation for IRIS assessments: (1) a literature survey to identify the possible health outcomes associated with the chemical, (2) construction of a table to guide the formulation of specific questions that will be the subject of specific systematic reviews, and (3) examination of this table to determine which health outcomes warrant a systematic review and to define the systematic-review questions. As an example, the NRC provided the question, "Does exposure to chemical X result in neurotoxic effects?" In addition to identifying health outcomes for systematic review, the problem formulation section discusses key issues that the assessment will address.

This document begins with a brief background information on ethylbenzene, which will be the subject of an IRIS assessment. Next the three steps that the NRC suggested are presented along with the systematic-review questions and key issues.

Early public involvement should increase the quality and transparency of IRIS assessments. Accordingly, the IRIS program is releasing this document in anticipation of a public science meeting focused on identifying the scientific information available for this assessment. The IRIS program encourages the scientific community and the general public to participate in this meeting.

1. BACKGROUND

1.1. Production and Use

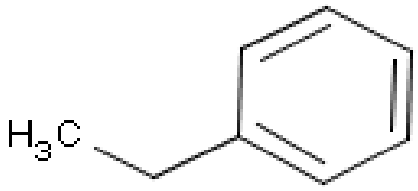

Figure 1. Chemical structure of ethylbenzene (NLM, 2005).

Ethylbenzene (CAS# 100-41-4), also known as phenylethane, is a colorless, flammable, and aromatic hydrocarbon that is present in crude petroleum and gasoline. In addition, it is used in industry primarily as a chemical intermediate in the production of styrene monomer (IPCS, 1996). Ethylbenzene has also been used as an industrial solvent and as a diluent in the paint industry as well as in the manufacture of synthetic rubber, acetophenone, and cellulose acetate (CalEPA, 1997). Ethylbenzene is present in naphtha, asphalt, and as an impurity in xylene solvents (CalEPA, 1997).

Ethylbenzene production volumes in the US range from 7-13 billion pounds per year, which is among the highest for chemicals manufactured in the US (ATSDR, 2010). The production and use of ethylbenzene in industry result in the potential for contamination of air, soil, and water (CalEPA, 1997). The presence of ethylbenzene in gasoline, as well as its use as a solvent, result in potential for release to air. Soil contamination may occur through fuel spillage, solvent disposal, or storage tank leakage. Water has the potential to become contaminated by ethylbenzene from industrial discharges, fuel spillage, leaking petroleum pipelines and underground storage tanks, landfill leachate, and improper disposal of wastes containing ethylbenzene.

According to the U.S. EPA's Toxics Release Inventory (TRI) Program, the environmental release of ethylbenzene in the US from facilities required to report in 2012 was approximately 2.7 million pounds into the atmosphere from fugitive emissions and point sources; 0.8 million pounds to land from landfills, land treatment, underground injection and other land disposal sources; and 4,531 pounds to surface waters (U.S. EPA, 2014). This is a decline of roughly 9.3 million pounds from the total release in 1994.

1.2. Environmental Fate

Ethylbenzene is not expected to be especially persistent in environmental media. With a K_{oc} value of 240, the mobility of ethylbenzene in soil is expected to be moderate. Volatilization from water and soil is likely to be an important environmental fate process for ethylbenzene, based on its vapor pressure (ATSDR, 2010). When released to the atmosphere, ethylbenzene is expected to exist predominantly in the vapor phase (ATSDR, 2010). In the atmosphere, ethylbenzene may adsorb to suspended particles and be removed along with the particles by precipitation or dry deposition (IPCS, 1996). The atmospheric half-life of gaseous ethylbenzene has been estimated at around 15 hours (IPCS, 1996).

Due to the contributions from tobacco smoke and attached garages, indoor air levels of ethylbenzene in residential settings are likely to be higher than outdoor levels, and have been reported to range from 1.00-110 μg/m^3 (ATSDR, 2010; U.S. EPA, 1987; U.S. EPA, 2010). Ethylbenzene air concentrations reported in occupational settings range from 365-2,340 μg/m^3 (ATSDR, 2010). Generally, ambient air concentrations of ethylbenzene are lower in rural areas than in urban areas, where vehicle emissions are thought to be a major contributor. ATSDR (2010) reports median levels of 0.62 ppb (2.7 μg/m^3) in urban and suburban locations and 0.01 ppb (0.056 μg/m^3) in rural locations.

Water has the potential to become contaminated by ethylbenzene from industrial discharges, boat fuel, and storage tank leakage. Thus, there is a higher potential for drinking water sources near leaking gasoline storage tanks to become contaminated (CalEPA, 1997).

1.3. Human Exposure Pathways

Individuals who are likely to have higher exposures are those living near hazardous waste sites where ethylbenzene has been detected or those using well water downgradient from leaking underground storage tanks. Ethylbenzene inhalation and ingestion estimates were higher in a household that used groundwater contaminated by gasoline from a leaking underground storage tank, compared to an unexposed cohort (ATSDR, 2010).

Inhalation is expected to be an important route of ethylbenzene exposure for the general population, particularly while pumping gasoline or driving, and by cigarette smoking. Median blood ethylbenzene levels prior to and after pumping gasoline were reported to be 0.10 μg/L and 0.16 μg/L, respectively (ATSDR, 2010). In the US, the median and 95th percentile blood levels are approximately 0.035 μg/L and 0.14 μg/L, respectively (CDC, 2013).

2. SCOPE OF THIS ASSESSMENT

A previous IRIS assessment on ethylbenzene was completed in 1991. Reference values were derived for oral and inhalation exposure. At that time, ethylbenzene was not classified in regard to its potential to cause cancer in humans due to a lack of animal and human data. Since then, a number of relevant studies on ethylbenzene toxicity have been conducted and new data are available. Ethylbenzene and naphthalene bioassays with mice have both resulted in lung tumors and raised similar questions of relevance to human health. An EPA workshop on mouse lung tumors associated with exposure to several compounds, including naphthalene and ethylbenzene, was conducted in January 2014. The IRIS program is evaluating these two chemicals simultaneously due to their having some similar toxicological issues.

Ethylbenzene has been identified as a concern at contaminated sites, as an air pollutant and a contaminant in drinking water. It has been listed under a number of environmental statutes that are implemented by EPA, including the Clean Water Act (CWA), Federal Insecticide Fungicide and Rodenticide Act (FIFRA), Clean Air Act (CAA), Safe Drinking Water Act (SDWA), Emergency Planning and Community Right-to-Know Act (EPCRA), Toxic Substances Control Act (TSCA), Resource Conservation and Recovery Act (RCRA), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). The chemical is on ATSDR's 2013 substance priority list.

A new IRIS assessment will evaluate all potential human health hazards associated with ethylbenzene exposure through oral and inhalation routes of exposure. An assessment for the dermal route of exposure is not planned at this point because oral and inhalation exposure are generally considered the major routes of exposure and evaluating risk from dermal exposure was not identified as a priority need. Furthermore, no dermal-only exposure studies in humans or experimental animals were identified.

3. PROBLEM FORMULATION

3.1. Preliminary Literature Survey

A preliminary literature survey was performed to identify health outcomes whose possible association with ethylbenzene has been investigated. This survey consisted of a search for health assessment information produced by other federal, state, and international health agencies, and an additional broad search of PubMed to locate more recent studies. The review of health assessment information results was used to narrow the list of potential health endpoints for consideration in the IRIS assessment and was supplemented by the PubMed search covering dates after the health assessments' publication. The PubMed search was not intended to be a comprehensive search of the available literature, but was intended to identify ethylbenzene health outcomes that had not been previously evaluated (*i.e.*, they were not a part of previous study designs) or were not observed in previous studies evaluated in prior health assessments. In addition, the preliminary literature survey was used to identify key scientific issues, including potential mode of action hypotheses that warrant evaluation in the assessment.

The following assessments, in addition to EPA's 1991 IRIS assessment (http://www.epa.gov/iris/subst/0051.htm), are available from several federal, state, and international health agencies (in reverse chronological order):

1. Occupational Safety and Health Administration. OSHA (2012). Chemical Sampling Information, Ethyl Benzene. https://www.osha.gov/dts/chemicalsampling/data/CH_240000.html
2. Agency for Toxic Substances and Disease Registry. ATSDR (2010). Toxicological profile for ethylbenzene. http://www.atsdr.cdc.gov/ToxProfiles/tp110.pdf
3. National Institute for Occupational Safety and Health. NIOSH (2010). NIOSH pocket guide to chemical hazards. RTECS. Benzene, ethyl-. http://www.cdc.gov/niosh/npg/npgd0264.html
4. California Environmental Protection Agency. Cal/EPA (2008). No significant risk levels (NSRLs) for the proposition 65 carcinogen ethylbenzene. http://www.oehha.ca.gov/Prop65/law/pdf_zip/EthylbenzeneNSRL032808.pdf
5. California Environmental Protection Agency. Cal/EPA (2007). Long-term health effects of exposure to ethylbenzene. http://oehha.ca.gov/air/hot_spots/pdf/Ethylbenzene_SRP082707.pdf

6. International Agency for Research on Cancer. IARC (2000). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 77, Some Industrial Chemicals. http://monographs.iarc.fr/ENG/Monographs/vol77/mono77-10.pdf
7. International Programme on Chemical Safety. IPCS (1996). Ethylbenzene. Volume 186. http://www.inchem.org/documents/ehc/ehc/ehc186.html

The additional PubMed search was limited to publication dates between November, 2010 and July, 2014 in order to identify studies released after the publication of ATSDR's 2010 Toxicological Profile for ethylbenzene (ATSDR, 2010). Search terms focused on each of the health outcomes shown in Table 1 and included a range of related terms. For instance, musculoskeletal effects search terms included ethylbenzene in conjunction with muscle, bone, muscular system, skeletal system, locomotion, locomotor system, cartilage, tendons, ligaments, or joints. All results of the PubMed search were screened by title and abstract to identify those appropriate for health assessment. The primary sources in the PubMed search included the following:

1. Billionnet C, Gay E, Kirchner S et al. 2011. Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings. Environ Res. 111(3): 425-434.
2. Martins PC, Valente J, Papoila AL et al. 2012. Airways changes related to air pollution exposure in wheezing children. Eur Respir 39(2): 246-253.
3. Wallner P, Kundi M, Moshammer H et al. 2012. Indoor air in schools and lung function of Austrian school children. J Environ Monit 14(7): 1976-1982.
4. Wang YR, Yand DY, Zhang M et al. 2011. The changes of blood neurotransmitter levels in workers occupationally exposed to ethylbenzene. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi [Chinese journal of industrial hygiene and occupational diseases]. 29(2): 125-127.
5. Zhang M, Wang Y, Wang Q et al. 2013. Ethylbenzene-induced hearing loss, neurobehavioral function, and neurotransmitter alterations in petrochemical workers. J Occup Environ Med 55(9): 1001-1006.
6. Zhang M, Wang YR, Yang DY et al. 2011. The neurobehavioral effects of population occupationally exposed to ethylbenzene. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi [Chinese journal of industrial hygiene and occupational diseases]. 29(2): 128-130.

3.2. Health Outcomes Identified by the Preliminary Literature Survey

The preliminary literature survey identified human, animal, and *in vitro* studies related to multiple health outcomes, mechanism of action, mode of action hypotheses, pharmacokinetics, and susceptible lifestages or subpopulations. Each row in Table 1 summarizes whether data are available on a particular health outcome or other toxicologically-relevant information, with each column indicating the types of studies that are available with respect to test system (human, animal, or *in vitro*) and exposure route (oral or inhalation, for *in vivo* studies). In addition, the table indicates whether animal studies of subchronic or chronic design are available, and whether the human studies are in an occupational, community, or clinical exposure setting. Studies that do not fall into any of these categories are indicated by checkmarks without an associated descriptor.

1 **Table 1. Ethylbenzene studies**

	Human Studies		Animal Studies		In Vitro Studies
	Oral	**Inhalation**	**Oral**	**Inhalation**	
Health Outcomes					
Body Weight Effects				✓ (Subchronic)	
Cancer		✓ (Occupational)	✓ (Chronic)	✓ (Chronic)	
Cardiovascular			✓ (Subchronic)	✓ (Subchronic, Chronic)	
Dermal				✓ (Chronic)	
Developmental				✓ (Subchronic)	
Endocrine				✓ (Subchronic, Chronic)	
Gastrointestinal				✓ (Subchronic, Chronic)	
Hematological		✓ (Occupational)	✓ (Subchronic)	✓ (Subchronic, Chronic)	
Hepatic			✓ (Subchronic)	✓ (Subchronic, Chronic)	
Immunological				✓ (Subchronic)	
Metabolic disease					
Musculoskeletal				✓ (Subchronic, Chronic)	
Neurological and Sensory		✓ (Occupational)	✓ (Subchronic)	✓ (Subchronic)	✓
Renal			✓ (Subchronic)	✓ (Subchronic, Chronic)	
Reproductive			✓ (Subchronic)	✓ (Subchronic)	
Respiratory		✓ (Community)	✓ (Subchronic)	✓ (Subchronic, Chronic)	
Other Data and Analyses					
ADME [1]		✓	✓	✓	
Toxicokinetic models [2]					✓
Mode of action hypotheses					✓
Susceptibility data		✓[3]			
Genotoxicity		✓	✓	✓	✓
Other mechanistic data					✓[4]

1 Absorption, distribution, metabolism and excretion (ADME) data also exists for dermal exposure for human and animals

2 Inhalation PBPKs included

3 Individuals that may be more susceptible to toxic effects include those with pre-existing hearing loss and diseases of the respiratory system, liver, kidney, or skin; fetuses; young children; pregnant women; and those taking certain medications, such as hepatotoxic medications or drugs (ATSDR 2010).

4 Adverse outcome models of carcinogenesis and benchmark dose

3.3. Hazard Questions for Systematic Review

The health agency reviews listed in Section 3.1 were used to "prescreen" end points considered most relevant for assessment and the effects noted in these reviews are summarized below. Based on the availability of health endpoint information indicated in Table 1, systematic reviews of the available literature are proposed for multiple endpoints, including: cancer, endocrine, hematological, immunological, hepatic, renal, neurological and sensory effects (including otological and ocular effects), respiratory, and reproductive and developmental effects. The summaries reflect characterizations provided by the other assessments and may differ from the final IRIS assessment's conclusions. The end points identified form the basis for developing the systematic review questions for a revised IRIS assessment. The systematic reviews would include analysis of available human, experimental animal, and *in vitro* studies. Systematic review questions were only developed where effects were noted.

Body Weight Effects

ATSDR (2010) identified transitory decreases in body weight gain in one study, while other studies show no changes in body weight.

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in body weight effects in humans?

Cancer

EPA's 1991 IRIS assessment classified ethylbenzene as "Group D – not classifiable as to human carcinogenicity" (U.S. EPA, 2011). Consequently, IARC (2000) classified ethylbenzene as "Group 2B – possibly carcinogenic to humans." The National Toxicology Program (NTP, 1999) conducted a two year inhalation study in rodents demonstrating increased incidence of renal tubule neoplasms in rats and an increased incidence of alveolar/bronchiolar neoplasms and hepatocellular neoplasms in mice. NTP (1999) indicated that there was clear evidence of carcinogenic activity of ethylbenzene in male F344/N rats based on increased incidences of renal tubule neoplasms. Additionally, the incidences of testicular adenomas were increased. NTP also determined that there was some evidence of carcinogenic activity of ethylbenzene in female F344/N rats based on increased incidences of renal tubule adenomas, in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms, and in female B6C3F1 mice based on increased incidences of hepatocellular neoplasms.

EPA's 1991 assessment of ethylbenzene found no studies suitable for determination of an oral qualitative or quantitative cancer value (U.S. EPA, 2011). CalEPA (2008) developed oral human cancer potencies based on the 1999 NTP inhalation study. No chronic oral ethylbenzene studies have been found in the literature.

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in carcinogenesis in humans?

Is ethylbenzene exposure associated with genotoxic and/or mutagenic effects related to its potential carcinogenicity? And if so, under what conditions?

Cardiovascular Effects

ATSDR (2010) identified a study which evaluated histologically the effects of ethylbenzene exposure on cardiac tissue. According to ATSDR (2010), no histological effects were noted in the study.

Dermal Effects

No dermal effects were identified following an inhalation exposure in rats or mice (ATSDR, 2010).

Developmental Effects

Increases in the incidence of extra ribs were noted in the offspring of rats exposed to ethylbenzene at various concentrations depending on the exposure period. In addition, significant reductions in fetal body weight have been observed (ATSDR 2010).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in developmental effects in humans?

Endocrine Effects

Long-term exposure to ethylbenzene has been shown to produce hyperplasia of the thyroid and pituitary glands (ATSDR 2010).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in endocrine effects in humans?

Gastrointestinal Effects

No adverse effects have been reported following subchronic and chronic inhalation of ethylbenzene in laboratory animals (ATSDR, 2010).

Hematological Effects

Studies in animals reporting hematological findings are unclear. One study reported significant decreases in platelet counts in female rats and significant increases in mean total leukocyte counts in male rats, while others report no effects. A decrease in platelet counts and an increase in mean corpuscular volume was noted in rats exposed orally for 13 weeks (ATSDR 2010). Wang et al. (2011) reported no significant difference in hematologic indexes including white blood cell, red blood cell, hemoglobin, and platelet counts in 246 workers occupationally exposed to ethylbenzene. In the same study it was reported that ethylbenzene decreased blood neurotransmitter (dopamine and acetylcholinesterase) levels in workers (Wang et al., 2011).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in hematological effects in humans?

Hepatic Effects

EPA's 1991 IRIS assessment (U.S. EPA, 2011) derived an oral reference dose based on liver and kidney toxicity from a 182 day gavage study in female rats reported in 1956. Since that time, a number of other studies have been identified by ATSDR (2010) as well as other agencies. A number of studies in laboratory animals have reported hepatic effects consistent with induction of microsomal enzymes (increase in liver weight, induction of hepatic drug metabolizing enzymes, and changes in the ultrastructure of the liver). Other effects include moderate to marked hypertrophy of the periportal hepatocytes, enlarged hepatocytes with multiple nuclei, hepatocellular hypertrophy and necrosis, and eosinophilic foci. A gavage study (13 week) in rats showed an increase in serum liver enzymes, increased absolute and relative liver weights, and increased incidence of centrilobular hepatocyte hypertrophy. In another study, increased liver weight and cloudy swelling of the parenchymal liver cells were noted in rats exposed for 6 months (ATSDR 2010).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in hepatic effects in humans?

Immunological Effects

Absolute and relative spleen weights were increased in pregnant rats during pre-mating and gestation or gestation alone, however no histopathological changes were noted (ATSDR 2010).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in immunological effects in humans?

Metabolic Disease

No studies were identified that evaluated the effects of ethylbenzene on metabolic diseases (ATSDR, 2010).

Musculoskeletal Effects

No musculoskeletal effects have been reported in laboratory animals following subchronic or chronic inhalation exposures.

Neurological and Sensory Effects

Acetylcholinesterase activity was significantly decreased ($p < 0.05$) in ethylbenzene-exposed petrochemical workers compared to control (office personnel). A negative correlation was also shown between acetylcholinesterase and neurobehavioral function (Zhang et al., 2013).

Neurobehavioral function: Scores of neurobehavioral function relating to memory and learning were significantly decreased ($p < 0.05$) in petrochemical workers compared to control (office personnel) (Zhang et al., 2013). According to Zhang et al. (2011), score of emotion, or vigor, was significantly lower ($p < 0.05$), while scores of fatigue and mean reaction time were significantly higher for occupationally exposed workers compared to control. It was also stated that scores of digital span, manual dexterity, visual retention and target tracking were significantly decreased compared to control (office workers). Furthermore, it was observed that for several neurobehavioral endpoints workers exposed to ethylbenzene for three years or longer differed significantly from workers exposed to ethylbenzene for 2 years or less, which suggests that workers exposed for three years to ethylbenzene may be a susceptible population of neurobehavioral function impairment (Zhang et al., 2011).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in neurobehavioral effects in humans?

Otological effects: Deterioration in auditory thresholds and alterations of cochlear morphology have been observed in laboratory animals exposed to ethylbenzene via inhalation (ATSDR 2010). Additionally, ethylbenzene-induced hearing loss has been observed in petrochemical workers. Hearing loss was significantly greater ($p < 0.05$) in ethylbenzene-exposed workers compared to groups exposed to noise and office personnel (Zhang et al., 2013).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in otological effects in humans?

Ocular effects: Eye irritation, a burning sensation, and profuse lacrimation have been observed in humans exposed to 1,000 ppm ethylbenzene. Ocular irritation and lacrimation have also been observed in rats, mice, and guinea pigs following acute exposure to ≥ 1,000 ppm ethylbenzene. Lacrimation was observed in rats exposed to 382 ppm for four weeks, while no ocular effects were documented in rats or mice after a 13-week exposure to 975 ppm ethylbenzene.

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in ocular effects in humans?

Renal Effects

EPA's 1991 IRIS assessment (U.S. EPA, 2011) derived an oral reference dose based on liver and kidney toxicity from a 182 day gavage study in female rats reported in 1956. Since that time, a number of other studies have been identified by ATSDR (2010) as well as other agencies. Exposure to ethylbenzene results in renal effects including increases in kidney weights, induction of drug metabolizing enzymes, accumulation of alpha 2u-globulin, nephropathy, renal tubule hyperplasia, and renal carcinogenesis. An increase in hyaline droplet nephropathy was noted in rats exposed orally for 13 weeks. A different study found increased kidney weight and cloudy swelling of the kidney tubular epithelium in rats exposed for 6 months (ATSDR 2010).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in renal effects in humans?

Reproductive Effects

No adverse effects on reproduction were observed following ethylbenzene exposure in laboratory animals (ATSDR, 2010). Rare histological changes had been reported but most studies are negative.

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in reproductive effects in humans?

Respiratory Effects

Human studies have reported throat and nasal irritation and a feeling of chest constriction during brief inhalation exposures and the severity of the symptoms increased with increased concentration. Subchronic to chronic inhalation studies in animals have reported no histopathological findings of the respiratory tissue (ATSDR, 2010). More recent studies have shown a negative association between ethylbenzene exposure and forced expiratory vital capacity, or FVC, (Wallner et al., 2012) and forced expiratory volume in one second, or FEV(1), in children (Wallner et al., 2012; Martins et al., 2012). Increasing exposure to ethylbenzene was also associated with acidity of exhaled breath condensate in children (Martins et al., 2012). In a different study ethylbenzene was significantly associated with rhinitis, or inflammation of the mucous membrane of the nose (38.3%) (Billionnet et al., 2011).

Systematic review question: Integrating the human, animal, and mechanistic evidence, what is the potential for ethylbenzene exposure to result in respiratory effects in humans?

3.4. Key Issues

Toxicokinetics of Ethylbenzene

The absorption, distribution, metabolism and excretion (ADME) of ethylbenzene have been reviewed by ATSDR (2010). Briefly, ethylbenzene is readily absorbed from a variety of exposure routes and is rapidly cleared from the blood in 60 minutes or less. Inhaled ethylbenzene accumulates in adipose tissue with concentrations of ethylbenzene in mesenteric adipose being 20-60 times higher than blood concentrations at steady state. The metabolism of ethylbenzene is mainly through hydroxylation and subsequent conjugations. Qualitative and quantitative metabolic differences exist between humans and laboratory animals and these differences may ultimately provide a basis for defining the relevance of adverse ethylbenzene endpoints in humans, but to date, mechanistic data have been lacking.

Studies are available comparing the rate and extent of metabolism of ethylbenzene in different tissues and in different animal species; and these are important for evaluating differences

across tissues and across species in ethylbenzene-related toxicity. For instance, lung specific expression patterns of cytochrome P450 enzymes, particularly CYP2F, have been investigated as potential explanations for differences in respiratory tract toxicity and cancer. In human tissues (based on *in vitro* metabolism studies of liver microsomes) other enzymes may be involved. Overall, inter- and intraspecies differences in metabolism could impact the extrapolation of rodent bioassay data to humans and the identification of potential susceptible subpopulations.

Based on the available data, some key issues EPA will evaluate regarding the toxicokinetics of ethylbenzene include:

- The chemical form (ethylbenzene or a metabolite) responsible for the various toxicities reported.
- Available information on inter- and/or intraspecies differences in the toxicokinetics relevant to ethylbenzene or its metabolites.
- The availability, evaluation, and further development (within assessment resources and time constraints) of PBPK models for reliable route-to-route, interspecies, and/or intraspecies extrapolation.

Mode of Action for Carcinogenicity

Rat kidney tumors

While rat renal tumors have been reported following exposure the ethylbenzene, there are varying perspectives on whether humans could be expected to develop the same type of tumor. Controversy exists around the MOA of these tumors and whether or not they are related to chronic progressive nephropathy (CPN); an age related condition found in rats, or are the tumors from a different mechanism. In 2002 Hard (2002) reevaluated the NTP histological findings and concluded that the increased incidence of renal tubule tumors was related to chemically-induced exacerbation of chronic progressive nephropathy (CPN), suggesting that because humans do not show a similar age-related renal pathology, these rat tumors are not relevant to humans. Seely et al. (2002) analyzed the association between CPN and renal tubule neoplasms in male F344 rats and concluded that the association between the two was marginal. Hard et al. (2012) expanded on the reanalysis by examining all control rats from 24 long-term NTP studies and concluded that advanced stages of CPN represent a risk for the development of a low incidence of renal tubule adenomas.

Mouse lung tumors

Mouse lung tumors following ethylbenzene inhalation have been investigated by several authors seeking to define the mode or modes of action (MOA) (Cruzan et al., 2009; Chan et al., 1998; Saghir et al., 2009; Saghir et al., 2010; Stott et al., 2003). The relevance of chemically-induced mouse lung tumors to human health has not been determined. While humans can develop lung

tumors, differences in the types of tumors, their location, metastatic propensities, cell of origin, and cell metabolism can affect the relevance of mouse lung tumors to human health.

Because of the importance of evaluating all existing information on this topic, recently EPA conducted a "State-of-the-science workshop on chemically-induced mouse lung tumors: applications to human health assessment" on January 7-8, 2014, RTP, NC. The focus of this workshop was to discuss the available data and interpretation of results from studies of mouse bronchiolar-alveolar adenomas and carcinomas (lung tumors) following exposure to naphthalene, styrene, or ethylbenzene, and the relevance of such tumors in mice to human cancer risk. Several panels of scientists discussed the available studies of human cancer epidemiology and pathophysiology, comparative pathology, biological mechanisms and evidence for cellular, genetic and molecular toxicology. The panelists included experts from academia, industry, government and nongovernmental organizations. The aim of the workshop was not to have the panel reach consensus on any particular topic, but to foster discussion across the different areas of expertise and viewpoints so that both EPA and the public could become better informed of the issues. Workshop materials can be obtained at http://www.epa.gov/iris/irisworkshops/mltw/. The workshop materials and topics discussed during this meeting will be used to inform the development of the ethylbenzene assessment. In addition, another similar workshop was conducted recently by the Styrene Information and Research Center to highlight mode of action research related to mouse lung tumors and human relevance (http://styrene.org/2013-mode-of-action-workshop).

Evaluation of Potential Mutagenic Mode(s) of Action

Ethylbenzene research and workshops have evaluated and discussed the potential for certain ethylbenzene metabolites, *e.g.*, 2,5-ethylquinone and 3,4-ethylquinone, to be mutagenic or exhibit other types of genotoxicity. The comparative metabolism of mice versus humans may inform the relevance of mouse tumors to potential human carcinogenesis. It is expected that the ethylbenzene re-assessment will require interpretation and analysis of mode of action research to inform the relevance of the observed ethylbenzene-induced mouse lung tumors.

The IRIS Program follows the Supplemental Cancer Guidelines (U.S. EPA, 2005b) that recommend an analysis of the available data for all carcinogenic chemicals to determine whether a mutagenic mode of action may be operational. This recommendation stems from a determination by the Agency that there is increased susceptibility for cancer when exposures occur early in life. If it is determined that ethylbenzene has human cancer potential by the oral or inhalation routes of exposure, then a specific determination regarding the mode of action as per the Supplemental Cancer Guidelines will be made.

Key issues related to mode of action for carcinogenicity

Based on the available data, the key issues for ethylbenzene mode of action for carcinogenesis include (but are not limited to):

- Identification of key events leading to the development of tumors in rats (kidney) and mice (lung)
- Role of metabolites in ethylbenzene-induced tumors
- Role of genotoxicity or mutagenicity in the mode of action of ethylbenzene-induced tumors
- Role of cytotoxicity and sustained regenerative cell proliferation in the mode of action of ethylbenzene-induced tumors
- Role of cytochrome P-450 enzymes in the development of lung tumors
- Role of CPN in the development of kidney tumors
- Species differences in enzyme activities and ethylbenzene toxicity

Based on the U.S. EPA (2005a,b) Cancer Guidelines framework for evaluation of mode of action, the following will be considered after a systematic review:

- Identification of mode of action hypotheses to be considered in the assessment
- Identification of the key events for each hypothesized mode of action
- Evaluation of experimental support for each hypothesized mode of action
- Sufficient support for each hypothesized mode of action in test animals
- Human relevance of hypothesized modes of action
- Populations or lifestages that are particularly susceptible to each hypothesized mode of action

Mechanisms of neurotoxicity, including ototoxicity

Studies have also demonstrated that ethylbenzene may exert detrimental effects on animal (Tegeris and Balster, 1994; Ethylbenzene Producers Association, 1986; Molnar et al., 1986; Cragg et al., 1989), as well as human (Yant et al., 1930) central nervous systems. In vivo studies of ethylbenzene toxicity in animals indicate that alterations in dopamine levels and other biochemical changes in the brain, as well as in evoked electrical activity in the brain may play a role in nervous system ethylbenzene-induced toxicity (Andersson et al., 1981; Frantik et al., 1994; Mutti et al., 1988; Romanelli et al., 1986).

Various in vitro studies on the mechanism of ethylbenzene induced-toxicity have paid particular attention to the chemical's effect on cell membranes, especially that of the astrocyte (Vaalavirta and Tahti, 1995a, 1995b; Sikkema et al., 1995; Naskali et al., 1993; Engelke et al., 1993). According to Sikkema et al. (1995), alterations in cell membrane integrity and structure following partitioning of ethylbenzene in to the lipid bilayer is a potential mechanism of toxicity. Additionally, as an in vitro model for the membrane mediated effects of solvents on the central nervous system, various studies have investigated ethylbenzene's effect on the membrane of rat astrocytes

(Vaalavirta and Tähti, 1995a, 1995b; Naskali et al., 1993, 1994). Cultured astrocytes of the cerebella were sensitive to ethylbenzene's effects, measured by inhibition of Na+, K+-ATPase, and Mg++-ATPase (Vaalavirta and Tähti, 1995a, 1995b). The effect was determined to be dose-dependent (Naskali et al., 1994). Perhaps, the cells' ability to maintain homeostasis is disrupted by inhibition of membrane-bound enzymes, which regulate membrane ion channels (ATSDR, 2010)

Animals have shown persistent hearing deficits following the cessation of ethylbenzene exposure and a recovery period, but it is unknown if humans would respond in a similar fashion. The slow or lack of recovery observed in animals could have significant health effect implications for humans exposed to ethylbenzene. The mechanisms of ototoxicity due to ethylbenzene exposure remain unclear; however, an in vitro study has suggested that low concentration ethylbenzene-induced ototoxicity may be mediated via nicotinic acetylcholine receptors. Under conditions of low receptor occupancy, ethylbenzene inhibited acetylcholine-mediated ion currents in human heteromeric α9 α10 nicotinic acetylcholine receptors, which were expressed in *Xenopus* oocytes (van Kleef et al, 2008).

Based on the available data, some key issues EPA will evaluate regarding the neurotoxicity and ototoxicity of ethylbenzene include:

- Reversibility, persistence and potential for progression of the neurobehavioral effects after humans are removed from ethylbenzene exposure
- Reversibility of the ototoxic effects in humans removed from ethylbenzene exposure
- The relevance of ototoxicity to humans at lower exposure levels

Human Susceptibility

Human susceptibility has already been discussed above in the context of toxicokinetics and mode of action. No other potential susceptibility factors have been identified for the toxic effects of ethylbenzene.

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