

**VOLUNTARY CHILDREN'S CHEMICAL EVALUATION
PROGRAM (VCCEP)**

TIER 1 PILOT SUBMISSION FOR

ETHYLBENZENE

(CAS No. 100-41-4)

Sponsors:

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The Dow Chemical Company

GE Plastics

INEOS Styrenics (formerly BP Amoco Chemical Company)

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REVISED AUGUST 10, 2007

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ACRONYMS AND ABBREVIATIONS

°C	degrees Celsius
°F	degrees Fahrenheit
µg/L	microgram per liter
µg/m ³	microgram per cubic meter
µg/mg	micrograms per milligrams
ABS	Acrylonitrile-Butadiene-Styrene
ACGIH	American Conference of Governmental Industrial Hygienists
AEGL	Acute Exposure Guideline Level
AFC	antibody-forming cell
AQS	Air Quality System
atm-m ³ /mol	atmosphere cubic meter per mole
ATSDR	Agency for Toxic Substances and Disease Registry
AUC _∞	area under the blood concentration vs. time curve, extended to infinite time
BASE	Building Assessment Survey and Evaluation
BCF	bioconcentration factor
BDL	below detection limit
BTEX	Benzene, toluene, ethylbenzene, xylenes
bwt	body weight
CAA	Clean Air Act
CAP	Compound Action Potential
CAS	Chemical Abstract Service
CEP	Complete Exposure Pathway
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CHO	Chinese hamster ovary
cm ²	square centimeters
CPN	Chronic progressive nephropathy calcinosis-Raynaud's phenomenon-oesophageal dismobility-sclerodactylytelangiectasia
CREST	syndrome of scleroderma
CWA	Clean Water Act
DPOAE	distortion product otoacoustic emissions
EB	Ethylbenzene
EC50	theoretical concentration producing an effect in 50 percent of the population
ECB	European Chemicals Bureau
EPA	Environmental Protection Agency
EPCRA	Emergency Planning and Community Right to Know Act
EPS	Expandable polystyrene
ETS	Environmental tobacco smoke
FCS	food contact substances
FDA	Food and Drug Administration
FDCPMC	Food, Drug and Cosmetic Packaging Material Committee
F ₀	parental generation
F ₁	first generation of offspring
F ₂	second generation of offspring
FOB	functional observational battery
ft ²	square feet
g/mol	gram per mole
gm/mL	gram per milliliter
GPPS	general purpose polystyrene
HAP	hazardous air pollutant
HazDat	Hazardous Substance Release/Health Effects Database

HI	hazard index
HIPS	high-impact polystyrene
HPV	high production volume
HQ	hazard quotient
hrs/day	hours per day
hrs/e	hours per event
HSDB	Hazardous Substance Database
HUD	Housing and Urban Development
ICRP	International Commission on Radiological Protection
IPCS	International Program on Chemical Safety
IRIS	Integrated Risk Information System
IUCLID	International Uniform Chemical Information Database
I/O	indoor/outdoor
K	volatilization factor
kg	kilogram
K_{oc}	Organic Carbon Partition Coefficient
K_{ow}	Octanol-Water Partition Coefficient
LCT	Leydig cell tumor
LD	lactation day
L/m^3	liter per cubic meter
m^2	square meter
m^3/day	cubic meter per day
m^3/hr	cubic meter per hour
MACT	Maximum Achievable Control Technology
MAFF	UK Ministry of Agriculture, Fisheries, and Food
MAK	Maximum Allowable Concentration
MCL	Maximum Contaminant Level
mg/day	milligram per day
mg/kg-day	milligram per kilogram per day
mg/L	milligrams per liter
mg/m^3	milligram per cubic meter
mmHg	millimeter of mercury
MRL	Minimal Risk Level
MSAT	Mobile Source Air Toxic
NAC	National Advisory Committee
NAWQA	National Water Quality Assessment
NCE	normochromatic erythrocyte
NCOD	National Contaminant Occurrence Database
NEI	National Emissions Inventory
NESHAP	National Emission Standards for Hazardous Air Pollutants
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOES	National Occupational Exposure Study
NPL	National Priority List
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration
PAMS	Photochemical Assessment Monitoring Stations
PBPK	Physiologically based pharmacokinetic
PCE	Polychromatic erythrocyte

PEL	Permissible Exposure Level
PES	Unsaturated polyester resins
PND	Postnatal day
PPA	Pollution Prevention Act
ppb	parts per billion
ppm	parts per million
PVC	polyvinyl chloride
PSWG	Polystyrene Work Group
RCRA	Resource Conservation and Recovery Act
RD50	Theoretical concentration producing 50 percent depression in respiratory rate
REL	Recommended Exposure Limit
RfC	Reference Concentration
RfD	Reference Dose
RFG	Reformulated Gasoline
RS	Robust Summary
RTG	Relative Growth
SACK	Sack Database
SAN	Styrene-acrylonitrile
SARA	Superfund Amendment and Reauthorization Act
SBL	Styrene-butadiene latex
SBR	Styrene-butadiene rubber
SDWA	Safe Drinking Water Act
SEBA	Styrene and Ethylbenzene Association
SHE	Syrian hamster embryo
SHIELD	School Health Initiative: Environment, Learning, Disease
SIAM	SIDS Information Assessment Meeting
SIAR	SIDS Initial Assessment Report
SIC	Standard Industrial Classification
SIDS	Screening Information Data Set
SML	Specific migration limit
SPI	Society of Plastics Industry
SRC	Syracuse Research Corporation
STEL	Short-term Exposure Limit
STORET	Storage and Retrieval Database
TDS	Total Diet Study
TEACH	Toxic Exposure Assessment, a Columbia/Harvard study
TEAM	Total Exposure Assessment Methodology
TLAEL	Theoretical lowest adverse exposure level
TLV	Threshold Limit Value
TRI	Toxic Release Inventory
TSCA	Toxic Substances Control Act
TWA	Time weighted average
UATMP	Urban Air Toxics Monitoring Program
UCL	upper confidence limit
UDS	Unscheduled DNA synthesis
UPR	Unsaturated polyester resins
U.S. EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
USPE	Unsaturated polyester resin
VCCEP	Voluntary Children's Chemical Evaluation Program
VCF	Volatile Compounds in Food
VOC	Volatile Organic Compound
yr	Year

TIER 1 ASSESSMENT FOR ETHYLBENZENE

1.0 EXECUTIVE SUMMARY

1.1 Introduction

The Voluntary Children's Chemical Evaluation Program (VCCEP), announced in December 2000, is an important component of the United States Environmental Protection Agency's (EPA's) Chemical Right-to-Know initiative. The stated purpose of the program is to provide the Agency and the public with the means to understand the potential health risks to children and prospective parents associated with their exposure to chemicals commonly found in human tissues and fluids (fat, blood, breath, milk, urine), as well as in dietary or consumer items and environmental media, so that exposure mitigation measures may be taken, as appropriate.

Ethylbenzene (Chemical Abstract Service [CAS] RN 100-41-4) was selected for the VCCEP Pilot Program because it was detected in human blood by the National Health and Nutrition Examination Survey (NHANES) and in expired air by the Total Exposure Assessment Methodology (TEAM) monitoring programs, it was detected in environmental media, and hazard data are available. In June 2001, the American Chemistry Council Ethylbenzene Panel volunteered to participate in VCCEP and to sponsor a Tier 1 assessment of ethylbenzene.

1.2 Production and Use

Ethylbenzene has two distinct chains of commerce. It is listed as a high production volume (HPV) chemical, with production of 14,395 millions of pounds produced per year (as of 2002). The majority of ethylbenzene is manufactured in reactions of benzene with ethylene. There have been as many as eleven different manufacturers of ethylbenzene in the United States (U.S.) with eight currently active. This product source and market chain is referred to within this report as the "ethylbenzene/styrene chain of commerce" and is the chain of commerce of primary interest for this evaluation.

Ethylbenzene is used primarily in the production of styrene and styrenic products. Styrene is an intermediate in the production of a number of commercially important polymers and copolymers: polystyrene, styrene-butadiene rubber (SBR), styrene-butadiene latexes (SBL), acrylonitrile-butadiene styrene (ABS), and styrene-acrylonitrile (SAN); unsaturated polyester resins; and miscellaneous products. These materials are used to make many products of industrial, consumer, and medical importance. Commercially important styrenic polymers may contain residual amounts of ethylbenzene from the production process. Ethylbenzene has also been used in the production of other industrial chemicals. It has been used in the manufacture of acetophenone, diethylbenzene, cellulose acetate, ethyl anthraquinone, ethylbenzene sulfonic acids, propylene oxide, and α -methylbenzyl alcohol.

Ethylbenzene also occurs as a natural component of petroleum products. In these products, ethylbenzene was not being produced for a specific purpose but was present due to the composition of the refinery hydrocarbon stream mixture. For example, gasoline typically contains about 2% ethylbenzene by weight. Ethylbenzene is also present as a component of hydrocarbon solvents, including commercial mixed xylenes, which may contain 6% to 15% of ethylbenzene by volume. As part of these mixtures, ethylbenzene may be found in diluents in varnishes, paints, and lacquers or found in solvents used in the rubber and chemical manufacturing industries. Ethylbenzene from these sources is termed in this document the “refinery chain of commerce.”

Additional sources of ethylbenzene are the incomplete combustion of natural materials (wood and other fuels) or from incinerators burning various hydrocarbon-containing waste streams. Ethylbenzene is also released in tobacco smoke either directly to the smoker or indirectly through environmental tobacco smoke (ETS).

1.3 Exposure Assessment

For ethylbenzene exposure to the general public through indoor air, outdoor air, and other source media, it was difficult to separate the contribution of the amount of ethylbenzene from the ethylbenzene/styrene chain of commerce, which is expected to be small, with that from the much larger refinery chain of commerce. Therefore, an additional objective of this assessment was to distinguish, on a semi-quantitative basis, that proportion of each exposure pathway that was directly attributable to the ethylbenzene/styrene chain of commerce. The proportion of ethylbenzene in ambient air that is attributable to the ethylbenzene/styrene chain of commerce cannot be precisely quantified, but a conservative estimate is thought to be 1%. Contribution of ethylbenzene attributable to the ethylbenzene/styrene chain of commerce to the population also exposed to ethylbenzene through smoking would be approximately 0.7%. The contribution of migration from food-contact material to the total dietary ethylbenzene concentration was conservatively estimated at 25%.

Estimated exposure concentrations in the identified media and population-specific exposure parameter values were used to estimate potential intake for children and prospective parents. Both the intake of ethylbenzene from exposure in the identified media from all sources of ethylbenzene and that portion of the total intake that could reasonably be attributed by ethylbenzene/styrene chain of commerce sources were considered. General intake equations for the inhalation pathway (due to exposure while at home, at school, outdoors, at work, and in a motor vehicle), dietary intake, ingestion of breast milk (for an infant), and mouthing of toys (for children) were used to estimate potential intake of ethylbenzene.

With children, with the exception of the age group <1 year, total intake by the inhalation pathway considered the amount of exposure at home, at school, outdoors, and riding in a motor vehicle. As expected, the contribution from the home represented 80% to greater than 90% of the total ethylbenzene intake by the inhalation route for all age groups. The inhalation pathway (the sum of microenvironments) was the most significant contributor to total ethylbenzene intake in all microenvironments. The percent contribution was, as expected,

greatest in the urban, smoking setting because the expected air concentrations, both outdoors and indoors, were the highest. The percent contribution was the lowest, as expected in the rural, non-smoking setting because the air concentrations were reduced but the contribution from the diet remained the same in each microenvironment.

Intake by way of the diet is a composite of the intake from all foods considered and was based on ethylbenzene concentrations in each food type and the age-specific ingestion rate for that food. The most significant contributor to diet for the bottle-fed infant was formula and whole milk, which were not assumed to be ingested by the breastfed infant. The contribution from breast milk for both the ethylbenzene worker's child and the nonworker's child was very small and the total estimated intake from diet was less than that estimated for a bottle-fed infant. As with the contribution from the diet, the air concentrations were lower in rural and non-smoking settings; therefore, the percent contribution from breast milk was higher but the absolute estimated intake did not differ. The daily exposure levels associated with toy mouthing were orders of magnitude lower than those associated with other exposure pathways. It was concluded that mouthing of styrenic toys is unlikely to be a significant source of children's exposure to ethylbenzene. For children, the total intake in all settings decreased with age, as expected because of the higher inhalation to body weight ratios for the younger age groups. The highest estimates of intake for the general public, as expected, were in the urban, smoking setting.

Key findings of the exposure assessment for children were that inhalation of ethylbenzene exceeded ingestion, urban exposures exceed rural/suburban exposures, and exposures of children ages 0-2 years old exceed those of children from age 3 to 19. The highest "central tendency" estimated intake was for bottle-fed infants <1 year old in an urban, smoking setting (3.63×10^{-3} mg/kg bwt/day total; 2.64×10^{-3} mg/kg bwt/day from inhalation, 9.90×10^{-4} mg/kg bwt/day from diet). The highest "upper-bound" intake estimate was for an ethylbenzene production worker's breastfed child (8.10×10^{-3} mg/kg bwt/day total; 5.87×10^{-3} mg/kg bwt/day from inhalation, 1.70×10^{-3} mg/kg bwt/day from breast feeding and 5.32×10^{-4} mg/kg bwt/day from diet).

Exposure pathways considered for prospective parents were inhalation of ethylbenzene - containing air in the workplace and other indoor, ambient, and motor vehicle environments and ingestion of food stuffs containing ethylbenzene. As with the higher end of the children age groups, inhalation was the dominant exposure pathway in the adult exposure scenarios, contributing at least 84% of the total intake. The influence of exposure setting on magnitude of adult exposure and relative contributions of the inhalation and ingestion pathways were similar to that discussed above for children. However, as expected, exposure for the production worker scenario was one to two orders of magnitude greater than those estimated for other adult populations due to the assumption of higher workplace exposure. The highest "central tendency" and "upper bound" exposures were for ethylbenzene production workers in an urban, smoking setting. These workers have negligible exposure to ethylbenzene from diet, as compared to inhalation exposure. The central tendency estimate of inhalation exposure for this group was 0.0229 mg/kg bwt/day, and the upper-bound estimate was 0.223 mg/kg bwt/day.

1.4 Hazard Assessment

1.4.1 Noncancer Effects

Ethylbenzene has low acute toxicity. Consistent with the known effects of organic solvents which cause a general and non-specific depression of the nervous system, acute exposure to high concentrations of ethylbenzene can induce acute neurological effects. Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies. Ethylbenzene is a moderate subchronic repeated exposure toxicity hazard by inhalation or oral dosing with consistent effects to the rodent liver and kidney. The subchronic oral study also detected a minimal regenerative anemia and a reduction in prothrombin time, both of questionable significance. Specialized investigations of ethylbenzene effects on hearing indicate inhaled ethylbenzene can cause ototoxicity. Ototoxicity has been reported in a recent 13-week study in rats that found alterations in brainstem auditory evoked responses and outer hair cell morphology in rats at concentrations of 200 ppm and greater ethylbenzene. Life-time inhalation exposures of ethylbenzene produced pathological lesions in the mouse liver, lung, thyroid, and pituitary gland. Rats that received lifetime exposures to ethylbenzene exhibited pathological changes to kidney, prostate gland, bone marrow, and liver. Ethylbenzene is not a teratogen or reproductive toxicant and is not (selectively) toxic to the developing nervous system. There is no evidence that ethylbenzene is harmful to the immune system.

1.4.2 Carcinogenicity

Ethylbenzene is carcinogenic in animals following lifetime exposures to high vapor concentrations. Tissue sites observed with increased tumor incidence following exposure to EB include the kidney (male and female rats), lung (male mice), and liver (female mice). Information regarding the cancer mode of action for each tissue site was examined within the context of the modified Hill criteria for causation. A direct mutagenic mode of action for EB is not supported by available information for any of the observed tumor types. Instead, EB appears to exert its carcinogenic effects via a nongenotoxic mode of action. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal cancer. Increases in regenerative cell proliferation, as a result of the formation of one or more reactive metabolites (catechols, quinones), are postulated to play a key role in the mouse lung tumor findings. Liver tumors in mice may be related to either a phenobarbital-like induction, which is not considered to be a relevant mode of action for humans, or as a result of the formation of one or more reactive metabolites (catechols, quinones).

1.5 Toxicity Reference Value Derivation

Existing noncancer reference concentration (RfC) and reference dose (RfD) values from U.S. EPA's Integrated Risk Information System (IRIS) were derived in 1991 and 1988, respectively. Currently, ethylbenzene is considered not classifiable as to human

carcinogenicity (Group D) by U.S. EPA (IRIS, 1991). Since that time, many additional studies pertaining to the toxicity, toxicokinetics, and potential mode of action (MOA) of ethylbenzene toxicity have been conducted. Proposed reference values that reflect the current state of knowledge regarding the cancer and noncancer effects were derived.

An RfC of 0.3 ppm, based on ototoxicity observed in rats, was used in this assessment. This proposed RfC is slightly higher than the existing RfC (0.2 ppm), but can be assigned greater confidence (medium-to-high confidence) than the existing IRIS RfC (low confidence).

An RfD of 0.5 mg/kg bwt/day is proposed based on liver effects observed in the chronic mouse inhalation study. The hepatic effects seen in the chronic mouse inhalation study and a subchronic oral rat study were similar. Use of the mouse inhalation study rather than the rat oral study obviates the need for an uncertainty factor for study duration (subchronic to chronic extrapolation) and increases confidence because the inhalation toxicity testing database is more extensive than the oral database. Overall, the confidence in the proposed RfD is medium-to-high.

A cancer reference value of 0.48 ppm (lower bound; central tendency = 0.80 ppm; and upper bound = 1.1 ppm) was derived for ethylbenzene based upon an uncertainty factor of 300 applied to the points of departure for mouse lung tumors, and applying a conservative estimate of human lung metabolism. These concentrations correspond to daily ingestion rates of 0.71 mg/kg bwt/day (lower bound; central tendency = 1.1 mg/kg bwt/day; and upper bound = 1.6 mg/kg bwt/day).

1.6 Risk Characterization

The risk characterization for ethylbenzene was performed using a hazard quotient (HQ) approach, as calculated using the following equation:

$$HQ = ADD / RfV$$

Where,

- HQ = Hazard quotient (unitless);
- ADD = Average daily dose, totaled for each route of exposure, and
- RfV = Reference value based upon noncancer or cancer endpoints.

HQs for the inhalation and ingestion routes of exposure were summed to calculate the hazard index (HI). An HI less than or equal to 1 is indicative that there is no elevated risk. The toxicity reference values and exposure estimates were used to assess the potential noncancer and cancer risks to children and adult populations exposed to ethylbenzene. None of the subpopulations considered in this risk assessment had a HI greater than 1. The risk characterizations for the most highly exposed groups are discussed in greater detail below.

Noncancer Risk Characterization

The central tendency estimates for bottle-fed urban infants (< 1 year old) in a smoking environment were HQs of 0.009 for inhalation and 0.002 for ingestion, for a total HI of 0.01. The upper bound estimates for a production worker's breast-fed infant in an urban, smoking environment were HQs of 0.02 for inhalation and 0.004 for ingestion, for a total HI of 0.02. These HIs indicate that even the most highly-exposed children are not at risk for noncancer effects of ethylbenzene.

The central tendency estimates for production workers living in an urban, smoking environment were HQs of 0.08 for inhalation and 0.0001 for ingestion, for a total HI of 0.08. The upper bound estimates for these workers were HQs of 0.7 for inhalation and 0.0003 for ingestion, for a total HI of 0.7. These HIs indicate that even the most highly-exposed prospective parents are not at elevated risk for noncancer effects of ethylbenzene.

Cancer Risk Characterization

The central tendency estimates for bottle-fed urban infants (< 1 year old) in a smoking environment were HQs of 0.004 for inhalation and 0.001 for ingestion, for a total HI of 0.005. The upper bound estimates for a production worker's breast-fed infant in an urban, smoking environment were HQs of 0.008 for inhalation and 0.003 for ingestion, for a total HI of 0.01. These HIs indicate that even the most highly-exposed children are not at risk for lung cancer from ethylbenzene exposure.

The central tendency estimates for production workers living in an urban, smoking environment were HQs of 0.03 for inhalation and 0.0001 for ingestion, for a total HI of 0.03. The upper bound estimates for these workers were HQs of 0.3 for inhalation and 0.0004 for ingestion, for a total HI of 0.3. These HIs indicate that even the most highly-exposed prospective parents are not at elevated risk for lung cancer from ethylbenzene.

1.7 Data Needs

Ethylbenzene has been evaluated in all the toxicity tests listed under Tier 1, Tier 2, and Tier 3 of the Pilot Announcement and overall this information is of suitable quality to support human health hazard and risk assessments for children and prospective parents. Additional investigation to further characterize the dose-response relationship between ethylbenzene and ototoxicity and the biological significance of certain measures of auditory response may be helpful to clarify hearing effects; however, the current VCCEP assessment has used a conservative interpretation of the biological significance of ototoxicity findings and hence no impact on the overall VCCEP assessment is anticipated from further ethylbenzene ototoxicity investigations.

The exposure assessment herein is adequate to describe current exposures for children and prospective parents. As ethylbenzene air concentrations in urban and suburban settings show steady declines (while rural concentrations remain steady), future exposure data are likely to be lower than the data used in this assessment, thus providing a conservative assessment of human health risk.

The risk assessment was conducted using EPA guidance. The calculated HIs indicate that even the most highly-exposed children and prospective parents are not at risk for noncancer or cancer effects of ethylbenzene. Therefore, further evaluations of risks of ethylbenzene under VCCEP are unnecessary.

2.0 BASIS FOR INCLUSION IN THE VCCEP PILOT PROGRAM

The Voluntary Children's Chemical Evaluation Program (VCCEP), announced in December 2000 (EPA, 2000a), is a component of the United States Environmental Protection Agency's (EPA's) Chemical Right-to-Know initiative. The stated purpose of the VCCEP is to provide the Agency and the public with the means to understand the potential health risks to children and prospective parents that may be associated with their exposure to chemicals commonly found in human tissues and fluids (fat, blood, breath, milk, urine) as a result of the chemical's presence in dietary or consumer items and/or environmental media (EPA, 2000b). If found, then exposure mitigation measures may be taken, as appropriate.

EPA's strategy for the VCCEP Pilot Program was to select chemicals that have been found in relevant biomonitoring and environmental monitoring databases and have sufficient hazard data available (EPA, 2000b). Ethylbenzene was selected for the VCCEP Pilot Program because ethylbenzene was:

- (1) detected in blood in the National Health and Nutrition Examination Survey (NHANES) (Ashley *et al.*, 1994) and in expired air in the Total Exposure Assessment Methodology (TEAM) (Wallace *et al.*, 1987) monitoring programs;
- (2) detected in air and groundwater monitoring programs (*e.g.*, Urban Toxics Monitoring Program [EPA, 2004a], Air Quality System [EPA, 2005a], National Water Quality Assessment [USGS, 2005], and National Contaminant Occurrence Database [EPA, 2005b]); and,
- (3) tested in studies that provided hazard data (a Screening Information Assessment Report [SIAR] available from the Organization for Economic Cooperation and Development [OECD] Screening Information Data Set [SIDS] Program [OECD, 2005]).

EPA characterizes the VCCEP at this time as a pilot program consisting of three tiers of assessment. Tier 1 is a screening evaluation, while Tiers 2 and 3, if considered necessary, more fully characterize potential exposure and risk, and may include additional data to resolve data gaps or significant uncertainties remaining after the screening assessment. Exposure assessment, hazard assessment, and risk characterization are the three key elements of a Tier 1 evaluation.

3.0 PREVIOUS ASSESSMENTS

This section summarizes previous assessments of human health effects of ethylbenzene. Assessments are listed in order of their preparation. The study interpretations noted below (e.g., study adequacy, identification of the No Adverse Effect Level [NOAEL]) reflect the opinions of the authors of the identified assessments, and may conflict with the interpretations the authors of this VCCEP assessment. These issues are addressed in the study summaries, hazard assessment, and risk characterization (see Appendices A and O and Sections 7 and 8).

3.1 U.S. EPA Integrated Risk Information System (IRIS) (1991)

The U.S. EPA's chronic health hazard assessments for the Integrated Risk Information System (IRIS) were last revised in (1991). The noncancer reference concentration (RfC) and reference dose (RfD) values from IRIS were derived in 1991 and 1988, respectively. The current RfD is 0.1 mg/kg bwt/day, based on liver and kidney effects (Wolf *et al.*, 1956). The current RfC is 1 mg/m³ (0.2 ppm) based on developmental effects in rats and rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981). In the IRIS carcinogenicity assessment, ethylbenzene was characterized as “not classifiable as to human carcinogenicity” due to a “lack of animal bioassays and human studies.” This chemical is currently being reassessed within the U.S. EPA IRIS program.

3.2 International Program on Chemical Safety (IPCS) (1996)

An Environmental Health Criteria report (No. 186) on ethylbenzene was prepared by the International Program on Chemical Safety (IPCS) (1996). A guidance value of 5 ppm was derived based on a No Observed Effect Level (NOEL) of 500 ppm and application of a composite safety factor of 100.

3.3 ATSDR (1999)

ATSDR (1999) derived a Minimal Risk Level (MRL) for inhalation exposures of intermediate duration of 1 ppm, based on developmental toxicity in rabbits (Andrew *et al.*, 1981). No acute inhalation MRL was derived due to lack of appropriate data. No chronic inhalation MRL was derived because the NOAEL from the study defining the intermediate MRL (100 ppm) was lower than the NOAEL for non-neoplastic effects in chronic studies (250 ppm [NTP, 1999]). No oral MRLs (acute, intermediate, or chronic duration) were derived for ethylbenzene due to lack of appropriate data.

3.4 International Agency for Research on Cancer (IARC, 2000).

Ethylbenzene was categorized as “possibly carcinogenic to humans (Group 2B)” (IARC, 2000).

3.5 OECD SIDS Initiative (2002)

The Screening Initial Data Set (SIDS) and SIDS Initial Assessment of Risk (SIAR) for ethylbenzene were reviewed at the 14th SIDS Information Assessment Meeting (SIAM 14),

March 26-28, 2002. The final report available online is dated May 2005 (OECD, 2005). Ethylbenzene was deemed to be a low priority for further work.

3.6 U.S. EPA Acute Exposure Guideline Levels (AEGLs)

The National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) was scheduled to discuss “various aspects of the acute toxicity and the development of Acute Exposure Guideline Levels (AEGLs)” for ethylbenzene and other chemicals at a September 6-8, 2006 meeting (Anonymous, 2006). As of November 27, 2006, no AEGLs for ethylbenzene had been established and no documentation of the September meeting was available on EPA’s AEGL site (www.epa.gov/opptintr/aegl/pubs/meetings.htm). The development of an AEGL for ethylbenzene is scheduled to be a topic of additional discussion at the NAC/AEGL meeting on December 12-14, 2006. An Interim AEGL-1 of 130 ppm for mixed xylenes (including almost 20% ethylbenzene) was used to evaluate acute exposure scenarios developed in the xylenes VCCEP submission (American Chemistry Council, 2005).

3.7 EU Risk Assessment

Ethylbenzene is on the First Priority list of the European Union, but no human health assessment has been prepared to date (European Chemicals Bureau [ECB], 2006).

3.8 Discussion

Ethylbenzene has been previously evaluated by several organizations. However, additional studies of relevant endpoints have been conducted since the preparation of many of the available assessments. In addition, some of the studies to derive toxicity reference values in these assessments are lacking in some respects, and considered inadequate for deriving toxicity reference values for the VCCEP program (see **Appendix A** and **Section 7 and 8**). Proposed toxicity reference values that reflect the current state of knowledge have been developed for use in the VCCEP assessment (**Section 8**).

4.0 REGULATORY OVERVIEW

This section provides an overview of the extensive federal environmental, health and safety, and related regulations applicable to ethylbenzene exposures. Ethylbenzene is broadly regulated by many federal agencies, including the U.S. Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), the Occupational Safety and Health Administration (OSHA), and the Department of Housing and Urban Development (HUD). Given the number, and in some cases, the complexity of these regulations, this overview necessarily is not an exhaustive survey of all regulations relating to ethylbenzene.

4.1 EPA Regulation

EPA regulates ethylbenzene under numerous statutes, including the Clean Air Act, 42 U.S.C. §§ 7401 *et seq.* (CAA); the Clean Water Act, 33 U.S.C. §§ 1251 *et seq.* (CWA); the Safe Drinking Water Act, 42 U.S.C. §§ 300f *et seq.* (SDWA); the Resource Conservation and Recovery Act, 42 U.S.C. §§ 6901 *et seq.* (RCRA); the Comprehensive Environmental Response, Compensation, and Liability Act, 42 U.S.C. §§ 9601 *et seq.* (CERCLA or Superfund), as amended by the Superfund Amendments and Reauthorization Act (SARA); the Emergency Planning & Community Right-To-Know Act (EPCRA), 42 U.S.C. §§ 11011 *et seq.*; the Pollution Prevention Act, 42 U.S.C. §§ 13101 *et seq.* (PPA); and the Toxic Substances Control Act, 15 U.S.C. §§ 2601 *et seq.* (TSCA).

4.1.1 Clean Air Act

The CAA regulates ethylbenzene emissions from stationary sources (*e.g.*, factories, refineries, and power plants) and mobile sources (*e.g.*, trucks, cars, motorcycles) and as volatile organic compounds (VOC) in products. Under the statute, ethylbenzene is variously referred to as a Hazardous Air Pollutant (HAP), a VOC, or a Mobile Source Air Toxic (MSAT).

4.1.1.1 Hazardous Air Pollutant Regulation

CAA Section 112 establishes a two-step process for protecting the public and the environment from the effects of air pollutant emissions considered hazardous from stationary sources. EPA first promulgates extensive National Emission Standards for Hazardous Air Pollutants (NESHAP), better known as Maximum Achievable Control Technology (MACT) standards, pursuant to CAA Section 112(d). These technology-based MACT standards are imposed on specific manufacturing sectors on a category-by-category basis. (*See generally* 40 C.F.R. Parts 61, 63.) Within the eight years following the promulgation of each technology-based MACT standard, EPA is required to regulate any remaining (or “residual”) risk with an “ample margin of safety” (CAA § 112(f), 42 U.S.C. § 7412(f)). In this second phase, EPA applies a risk-based approach to assess whether the MACT technology-based emission limits sufficiently reduce health and environmental risks. Ethylbenzene emissions from stationary sources are subject to both stringent, manufacturing sector-specific MACT-based standards and potential further regulation that may be determined necessary to ensure

an ample margin of safety. Virtually all of the MACT standards have already been promulgated, and EPA is in the process of considering where residual risk rules for facilities may be needed.

4.1.1.2 Volatile Organic Compound Regulations

Numerous regulations affect VOCs in regions where ozone formation is a concern. While these regulations are not necessarily specific to ethylbenzene, some may apply to industrial operations that emit ethylbenzene. (*See, e.g.*, 40 C.F.R. Part 60 (VOC standards for new stationary sources involving certain activities).) The overriding impact of these regulations is a further reduction of ethylbenzene emissions.

4.1.1.3 Mobile Source Air Toxics, Reformulated Gasoline, and Limits on Gasoline Volatility

According to EPA, “nationwide, mobile sources represent the largest contributor to air toxics.” (EPA, Mobile Source Emissions -- Past, Present, and Future.) The CAA requires EPA to promulgate regulations to control HAPs from motor vehicles and motor vehicle fuels. The regulations must reflect the greatest degree of emission reduction achievable, considering “the availability and costs of the technology, and noise, energy, and safety factors, and lead time” (CAA § 202(l)(2), 42 U.S.C. § 7521(l)(2)). As a result, numerous regulations reduce emissions of MSATs such as ethylbenzene, including EPA’s reformulated gasoline (RFG) program, limitations on gasoline volatility, and other provisions that affect MSATs.

Subsequent to the passage of the 1990 CAA Amendments, EPA established the RFG program, which requires the reformulation of gasoline to reduce emissions of smog-forming and toxic pollutants (*see generally* 40 C.F.R. Part 80). Other regulations limit gasoline volatility, thereby reducing evaporative emissions (*see, e.g.*, 40 C.F.R. § 80.27). Volatility is a measure of how easily gasoline evaporates. When gasoline evaporates, substances such as ethylbenzene that are present in the gasoline are released to the air. EPA regulates the Reid vapor pressure of gasoline, a common measure of gasoline volatility, from May through September each year for certain “designated volatility nonattainment areas” and “designated volatility attainment areas” as defined in 40 C.F.R. Section 80.2(cc) and 40 C.F.R. Section 80.2(dd), respectively. Moreover, certain classes of motor vehicles are required to have evaporative emission controls, thereby further reducing the amount of gasoline volatiles released into the air (*see, e.g.*, 40 C.F.R. §§ 86.1811-01(d), 86.1811-04(e), 86.1812-01(d), 86.1813-01(d), 86.1814-01(d), 86.1814-02(d), 86.1815-01(d), 86.1815-02(d), 86.1816-05(d), 86.1816-08(d)).

In 2001, EPA promulgated a MSATs final rule that identified 21 MSATs, including ethylbenzene, and set new gasoline toxic emission performance standards (*see* 66 Fed. Reg. 17230 (Mar. 29, 2001)). This rule establishes a framework for EPA’s national mobile source air toxics program and requires that refineries maintain the toxics performance of any gasoline produced during the baseline period 1998–2000. The rule also contains a plan for continuing research and analysis on all MSATs. In March 2006, EPA issued a proposed

mobile source air toxics rule designed to reduce emissions of ethylbenzene and other MSATs (*see* 71 Fed. Reg. 15803 (Mar. 29, 2006)). The proposed rule would lower emissions of ethylbenzene and other air toxics by lowering their content in gasoline, reducing exhaust emissions from passenger vehicles operated at cold temperatures (under 75 degrees Fahrenheit), and reducing emissions that evaporate from, and permeate through, portable gasoline containers (*i.e.*, gas cans).

4.1.2 Clean Water Act

The CWA, originally enacted as the Federal Water Pollution Control Act Amendments of 1972, establishes the basic structure for regulating discharges of pollutants into the navigable waters of the United States. It prohibits any person from discharging any pollutant from a point source into navigable waters except in compliance with the CWA's permit requirements, effluent limitations, and other relevant provisions. The CWA also grants EPA the authority to set wastewater standards for industry and water quality standards for all contaminants in surface waters.

Ethylbenzene has been designated a hazardous substance under the CWA (*see* 40 C.F.R. § 116.4). Because of this designation, discharges of ethylbenzene are regulated, and certain releases must be reported. Direct discharges of wastewater from sources using end-of-pipe biological treatment cannot exceed an ethylbenzene concentration of 108 µg/L on any particular day, and a monthly average of 32 µg/L (*see* 40 C.F.R. § 414.91). For indirect-discharge sources and direct-discharge sources that do not use end-of-pipe biological treatment, the maximum ethylbenzene concentrations are 380 µg/L daily and 142 µg/L monthly (*see* 40 C.F.R. §§ 414.101, 414.111). Releases in excess of 1,000 pounds of ethylbenzene from any facility must be reported to the National Response Center (*see* 40 C.F.R. § 117.3).

In addition, EPA has established water quality standards that vary by body of water, for states that do not comply with federal guidance for establishing their own standards under the CWA (*see* 40 C.F.R. §§ 131.31–40).

4.1.3 Safe Drinking Water Act

The SDWA creates a comprehensive scheme for regulating drinking water and its sources. Under the statute, EPA sets standards for approximately 90 contaminants in drinking water and its sources -- rivers, lakes, reservoirs, springs, and groundwater wells. For each of these substances, EPA sets an enforceable limit, called a maximum contaminant level (MCL), and a non-enforceable public health goal, called a maximum contaminant level goal (MCLG), which allows for a margin of safety.

EPA has set the MCLG for ethylbenzene in public drinking water sources at 0.7 mg/L, and the MCL at 0.7 mg/L (*see* 40 C.F.R. §§ 141.50, 141.61). The permissible level for ethylbenzene in bottled water products is 0.7 mg/L (*see* 21 C.F.R. § 165.110(b)(4)(iii)(B)).

In addition to MCLGs, MCLs, and other similar drinking water standards, EPA also promulgates health advisories, or guidance values, based on non-cancer health effects for different durations of exposure (*e.g.*, one-day, ten-day, and lifetime exposures). These health advisories provide technical guidance to EPA, state and local governments, and other public health officials regarding “health effects, analytical methodologies, and treatment technologies associated with drinking water contamination” (*see* description of U.S. EPA health advisories at <http://www.epa.gov/waterscience/criteria/drinking/>). EPA has issued at least one health advisory for ethylbenzene, establishing a level of 30 ppm (*see* Ethyl Benzene Health Advisory, Office of Water, EPA, EPA 820K87013 (March 1987)).

4.1.4 Resource Conservation and Recovery Act

RCRA regulates the transportation, treatment, storage, and disposal of hazardous wastes. Ethylbenzene and certain substances containing ethylbenzene are identified as F003 on the so-called F list of hazardous wastes, which includes hazardous wastes from non-specific sources (*see* 40 C.F.R. § 261.31). Materials that are destined for disposal that contain sufficient quantities of ethylbenzene may also be “characteristic” hazardous waste and be subject to regulation as a hazardous waste through this waste identification method as well. Ethylbenzene also is listed on the groundwater monitoring list for owners and operators of hazardous waste treatment, storage, and disposal facilities (*see* 40 C.F.R. Pt. 264, App. IX). Thus, ethylbenzene is subject to a variety of RCRA controls relating to its transportation, treatment, storage, and disposal.

4.1.5 Comprehensive Environmental Response, Compensation, and Liability Act

CERCLA, as amended by SARA, provides EPA with broad authority to respond directly to releases and threatened releases of hazardous substances, pollutants, and contaminants that are considered possible of endangering public health or the environment. Ethylbenzene has been designated as a hazardous substance under CERCLA (*see* 40 C.F.R. § 302.4). As a result, it is subject to monitoring and numerous other requirements relating to releases and threatened releases. For example, and as noted above, releases of ethylbenzene in excess of 1,000 pounds from any facility must be reported to the National Response Center (*see* 40 C.F.R. Part 302). In addition, certain amounts of other products containing ethylbenzene are reportable. Moreover, ethylbenzene present in contaminated media at listed Superfund sites is subject to varying levels of cleanup.

4.1.6 The Emergency Planning and Community Right-To-Know Act and The Pollution Prevention Act

Title III of SARA, also known as EPCRA, was enacted by Congress to help inform local communities of chemical hazards in their areas. EPCRA Section 313 requires EPA and state governments to collect data annually on releases and transfers of certain chemicals from industrial facilities. These data are available to the public through the Toxics Release Inventory (TRI). In 1990, Congress amended EPCRA reporting requirements by passing the PPA. Section 6607 of the PPA requires facilities to provide information on pollution

prevention and recycling for each chemical subject to reporting under the TRI (*see* 42 U.S.C. § 13106).

Ethylbenzene is one of the more than 650 chemicals and chemical categories subject to reporting under the TRI (*see* 40 C.F.R. § 372.65). Thus, users of ethylbenzene in many industries, such as petroleum refineries, manufacturers, miners, petroleum bulk terminals, and chemical wholesalers, are subject to these reporting requirements.

4.1.7 Toxic Substances Control Act

TSCA is the federal law that regulates new and existing chemical substances and provides a regulatory framework to address chemicals throughout their production, use, and disposal. Under TSCA, EPA classifies chemical substances as either “existing” or “new.” Existing chemicals are those listed on the TSCA Chemical Substance Inventory, or TSCA Inventory, which EPA must compile, keep current, and publish (*see* TSCA § 8(b), 15 U.S.C. § 2607(b)). Under TSCA, EPA is authorized to seek various kinds of health and safety data on existing chemicals, which include mandatory reporting under TSCA Section 8 (15 U.S.C. § 2607), and testing under TSCA Section 4 (15 U.S.C. § 2603).

4.2 FDA Regulation

FDA regulates a myriad of products ranging from food ingredients and drugs to medical and surgical devices. Only a sample of FDA’s regulations relating to ethylbenzene is discussed below.

In general, FDA limits the amount, if any, of ethylbenzene that can be contained in food and drugs. Ethylbenzene is not an approved food additive that can be added directly to food for human consumption (*see* 21 C.F.R. Part 172). Nor is ethylbenzene an approved substance for use in the food-contact surface of packaging for processing, transporting, or holding certain foods or for use in other food-contact surfaces (*see, e.g.,* 21 C.F.R. §§ 176.180, 177.1010). Ethylbenzene also is not approved for use in food packaging cellophane (*see* 21 C.F.R. § 177.1200). FDA limits the permissible amount of ethylbenzene in bottled water products to 0.7 mg/L (*see* 21 C.F.R. § 165.110(b)(4)(iii)(B)). Furthermore, FDA provides guidance on the amounts of residual solvents that are considered safe in pharmaceuticals, although ethylbenzene is not listed, except as a typical component of xylene (*see* FDA, Guidance for Industry, Q3C-- Tables and List at <http://www.fda.gov/cber/gdlns/ichq3ctablist.htm>).

4.3 OSHA Regulation

Under the Occupational Safety and Health (OSH) Act of 1970, OSHA is the federal agency responsible for establishing and enforcing workplace standards, including exposure limits for many substances. The National Institute for Occupational Safety and Health (NIOSH) and

the American Conference of Governmental Industrial Hygienists (ACGIH) develop and recommend exposure limits for worker protection, but these limits are not enforceable.

OSHA sets both permissible exposure limits (PEL) and short-term exposure limits (STEL). A PEL is the maximum concentration to which workers may be exposed in any 8-hour work shift of a 40-hour work week, and a STEL is the maximum 15-minute concentration to which workers may be exposed during any 15-minute period of the workday. The PEL for ethylbenzene is 100 ppm as an 8-hour time-weighted average (TWA) concentration.

NIOSH's recommended exposure limit (REL) for ethylbenzene is 100 ppm as a TWA for up to an 8-hour work shift and a 40-hour work week, and the recommended STEL is 125 ppm (*see* NIOSH Pocket Guide to Chemical Hazards). ACGIH has assigned ethylbenzene a threshold limit value (TLV®) of 100 ppm as a TWA for a normal 8-hour workday and a 40-hour work week, and a STEL of 125 ppm for periods not to exceed 15 minutes (*see* ACGIH, 2006 TLVs® and BEIs®).

4.4 HUD Regulation

HUD attempts to minimize exposure to ethylbenzene through regulations relating to the location of HUD-assisted projects. These regulations help calculate the acceptable separation distance between HUD-assisted projects and hazardous operations that store, handle, or process hazardous substances, and also provide guidance for identifying and assessing these hazardous operations. Ethylbenzene is one of the hazardous substances addressed by these regulations (*see* 24 C.F.R. Part 51, Subpart C, App. I).

4.5 State Regulation

In addition to the various federal regulatory programs described briefly above, ethylbenzene is subject to a wide variety of state regulations. A description of such programs is well beyond the scope of this regulatory overview, but in many instances, these regulatory programs are more stringent than federal requirements. Many federal statutes, such as the CAA and the OSH Act, permit or, in some instances, require states to apply additional regulatory measures. For example, California has extensive air toxics and VOC regulations that go well beyond federal requirements. These include specific air toxics programs, stringent mobile source (both fuels and vehicle) controls, and other regulatory controls. In recent years, many of these California programs have been adopted or extended by other states, particularly those in the Northeast. Ethylbenzene is on California's Proposition 65 list ("known to the State to cause cancer"). More recently, several localities have enacted local air toxics programs that provide further controls on releases of ethylbenzene to the environment.

5.0 CHEMICAL OVERVIEW

5.1 Product Overview

Ethylbenzene has at least two distinct chains of commerce. It is a by-product of petroleum refining and coal refining and occurs as a natural component of gasoline, other crude oil products, and a constituent of commercial xylene (“mixed xylenes”). Ethylbenzene from these sources is termed in this document as the “refinery chain of commerce”. Ethylbenzene is also an important industrial chemical used primarily in the production of styrene and styrenic products. Ethylbenzene from these sources is termed in this document as the “ethylbenzene/styrene chain of commerce”.

5.1.1 Production of Ethylbenzene

Ethylbenzene is listed as a high production volume (HPV) chemical with production exceeding one million pounds annually (OECD, 2005). The majority of commercial ethylbenzene is manufactured by the alkylation of benzene with ethylene or by the vapor-phase reaction of benzene with dilute feedstock containing ethylene (ATSDR, 1999). Other methods of manufacturing ethylbenzene include preparation from acetophenone, dehydrogenation of naphthenes, catalytic cyclization and aromatization, separation from mixed xylenes via fractionation, reaction of ethylmagnesium bromide and chlorobenzene, extraction from coal oil, and recovery from benzene-toluene-xylene processing (ATSDR, 1999; HSDB, 2004).

The ethylbenzene industry is closely tied to the styrene industry, because most of the ethylbenzene produced is used in the manufacture of styrene via hydrogenation of ethylbenzene and, to a much less extent, peroxidation of ethylbenzene with subsequent hydration (ATSDR, 1999). There have been as many as eleven different manufacturers of ethylbenzene in the United States (U.S.) (ATSDR, 1999). Currently there are eight producers of ethylbenzene in the U.S. These producers, along with their published annual mid-2002 production capacity, are listed in Table 5-1 (Ring and Inui, 2002).

As indicated in Figure 5-1, trends in U.S. production of ethylbenzene and styrene have been rising very slowly. From 1993 to 2003, the average annual changes in production were 2.8% for ethylbenzene and 1.7% for styrene (CEN, 2004).

5.1.2 Uses of Ethylbenzene

Ethylbenzene use in the United States can be assigned to two distinct chains of commerce. As indicated in Section 5.1.1, ethylbenzene is manufactured commercially and used in the production of styrene. The ethylbenzene/styrene chain of commerce uses are described in Section 5.1.2.1. Ethylbenzene is also present, along with a number of other constituents, in petroleum products and other chemical mixtures either as a naturally occurring component or a by-product. In these cases ethylbenzene is not being produced for a specific use but is included due to the makeup of the mixture. For this discussion, this type of use is referred to as the refinery chain of commerce (see Section 5.1.2.2). Other sources of ethylbenzene not directly related to either chain of commerce are discussed in Section 5.1.2.3.

Table 5-1 U.S. Producers of Ethylbenzene

Producer	Mid-2002 Annual Production Capacity (10 ⁶ pounds)
BP, Texas City, TX	1,130
Chevron Phillips Chemical Company LP, St. James, LA	2,150
Cos-Mar Company, Carville, LA	2,260
Dow Chemical U.S.A., Freeport, TX	1,750
Lyondell Chemical Company, Channelview, TX	3,245
NOVA Chemicals Corp., Bayport, TX	1,490
Sterling Chemicals, Inc., Texas City, TX	1,920
Westlake Styrene Corporation, Sulphur, LA	450
TOTAL	14,395
Source: Ring and Inui (2002).	

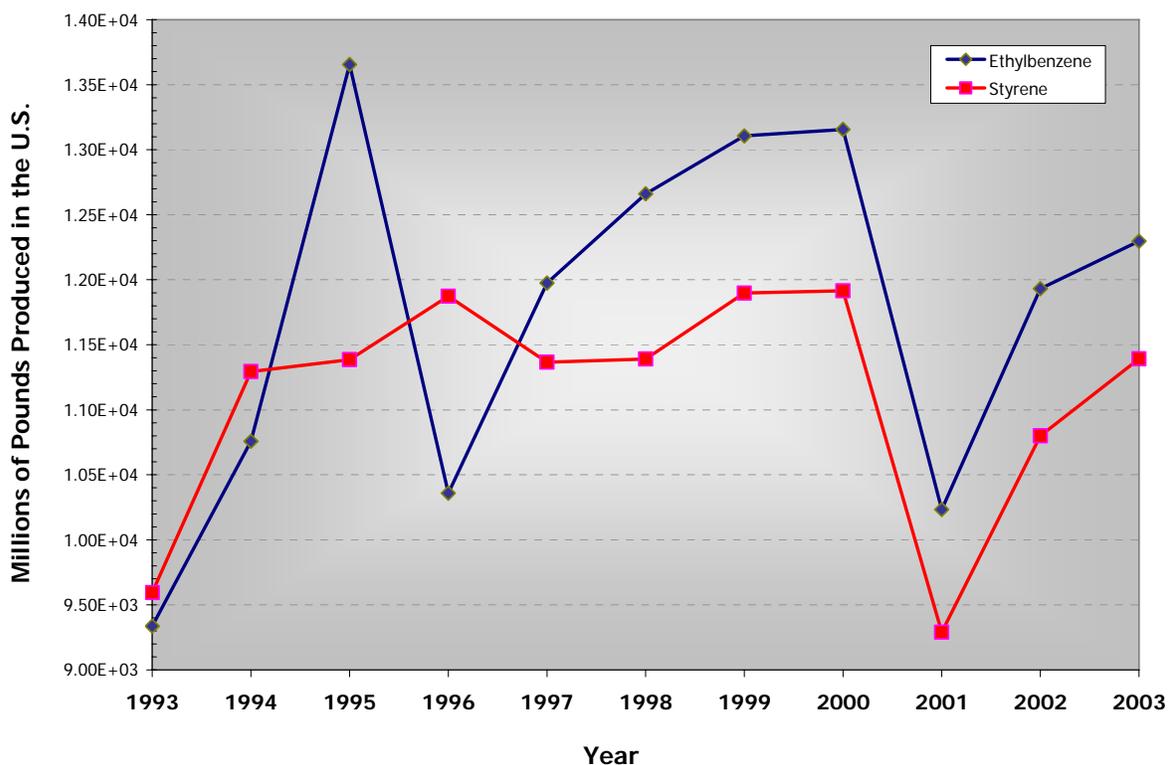


Figure 5-1. Trends in U.S. Production of Ethylbenzene and Styrene, 1993 – 2003 (data from CEN 2004)

5.1.2.1 Ethylbenzene/Styrene Chain of Commerce Uses

As indicated previously, nearly all the ethylbenzene produced in the U.S. is used for styrene synthesis. Styrene is an intermediate in the production of a number of commercially important polymers and co-polymers: polystyrene, styrene-butadiene rubber (SBR), styrene-

butadiene latexes (SBL), acrylonitrile-butadiene styrene (ABS) and styrene-acrylonitrile (SAN), unsaturated polyester resins, and miscellaneous products (EPA, 1993). The major producers of polystyrene, as of 2002, are indicated in Table 5-2.

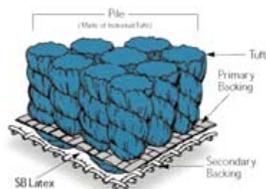
Table 5-2 U.S. Producers of Polystyrene, 2002

Producer	Production Capacity (10⁶ pounds)
American Polymers, Worcester, MA	75
American Polystyrene, Torrance, CA	30
ATOFINA-Petrochemicals, Carville, LA	1,125
BASF, Joliet, IL	760
BASF, South Brunswick, N.J.	300
Chevron Phillips Chemical, Marietta, OH	800
Dart Polymers, Owensboro, KY	105
Deltech, Troy, OH	150
Dow Chemical, Gales Ferry, CT	160
Dow Chemical, Hanging Rock, OH	200
Dow Chemical, Joliet, IL	280
Dow Chemical, Midland, MI	290
Dow Chemical, Pevely, MO	170
Dow Chemical, Torrance, CA	250
GE Plastics, Selkirk, NY	100
Huntsman, Peru, IL	260
Kama, Hazleton, PA	75
Nova, Beaver Valley, PA	280
Nova, Belpre, OH	485
Nova, Chesapeake, VA	450
Nova, Decatur, AL	380
Nova, Painesville, OH	70
Nova, Springfield, MA	300
StyroChem, Fort Worth, TX	120
TOTAL	7,215
Source: The Innovation Group (2004).	

Production of major styrenic-type resins for 2004 are shown in Table 5-3.

Table 5-3 Year-End Production for Styrenic Plastics for 2004^a

Resin	Production Volume (10⁶ Pounds)	Percent of Total Styrenics Produced
Polystyrene	6,744	68%
ABS	1,376	14%
SAN	132	1%
Other styrenics	1,716	17%
Total	9,968	
^a Source: American Plastics Council Plastics Industry Producers' Statistics Group (2004)		



These materials are used to make many products of industrial, consumer, and medical importance, as described below:

- **Polystyrene** -- the homopolymer of styrene, pure

polystyrene is referred to as crystal or general-purpose polystyrene (GPPS). To increase hardness, styrene may be polymerized in the presence of polybutadiene rubber to form rubber-modified or high-impact polystyrene (HIPS). Addition of a blowing agent to polystyrene results in expandable polystyrene (EPS). Thus, polystyrene products may be solid or foamed, and are widely used as packaging materials or containers for food, such as egg cartons, beverage cups, wrappings, trays, and other disposable



products. Polystyrene is also used in toys (*e.g.*, model cars and airplanes), recreational and sporting goods (*e.g.*, sports helmets, floating rings and pool toys), kitchen appliances, office furnishings and supplies, cabinets for consumer electronics, CD holders, cosmetic and personal care product containers, paper coatings, boat hulls, and interior automotive components (EPA, 1991). In the construction industry, polystyrene



materials are used to produce pipe products, tanks, lighting fixtures, insulation, and various corrosion resistant and rubber products.

- **Acrylonitrile-Butadiene-Styrene (ABS)** -- a tough, heat- and impact-resistant thermoplastic widely used for appliance and telephone housings, luggage, sporting helmets, pipe fittings and automotive parts.
- **Styrene-acrylonitrile (SAN)** -- a transparent, rigid plastic offering high chemical resistance, used mainly in the automotive, electrical and electronics industry, as well as in household applications and building products.
- **Styrene-butadiene latex (SBL)** -- a water-based polymer used for carpet backing and paper coating.
- **Styrene-butadiene rubber (SBR)** -- a high molecular weight polymer widely used in automobile and truck tires, belting, flooring, wire and cable insulation, footwear, and as a paper coating.



- **Unsaturated polyester resins (UPR, USPE or PES)** -- durable, resinous polymers used mainly in the construction, boat building, automotive and electrical industries. Usually reinforced with small glass fibers.

The estimated percent of world-wide styrene production used for these major resin families is shown in Figure 5-2.

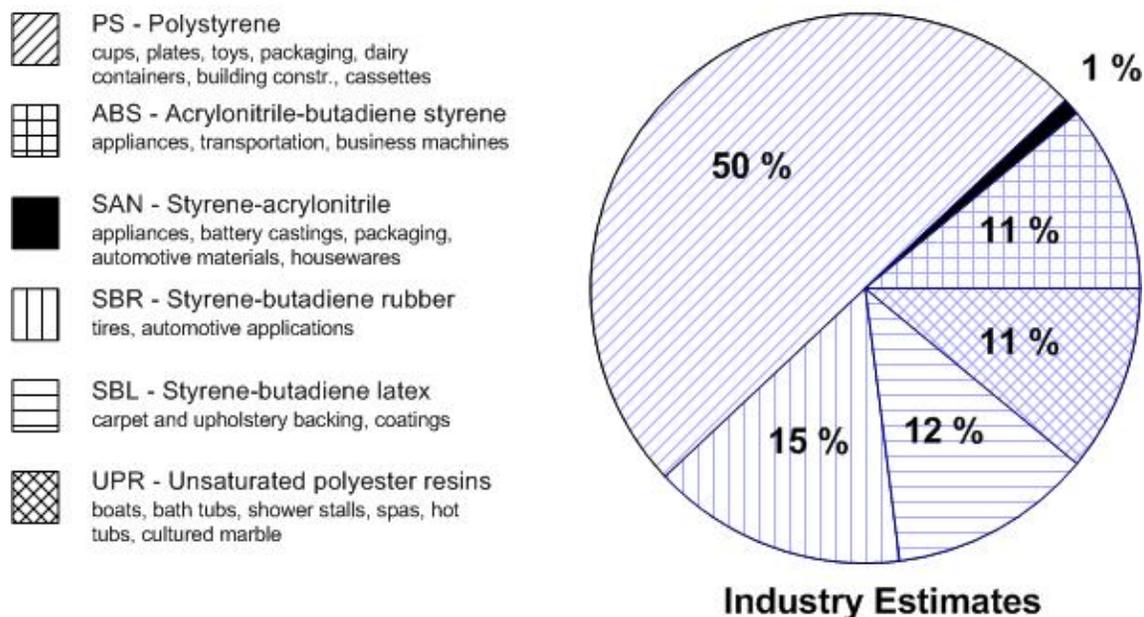


Figure 5-2 Six Major Styrene Resin Families (Source: The Styrene Forum [2005])

Commercially important styrenic polymers may contain residual amounts of ethylbenzene from the production process. For example, ethylbenzene was found in 41 of 44 samples of polystyrene products (median concentration was 50 mg/kg, with a range 8 – 473 mg/kg) and in all 12 samples of styrene graft and copolymer products (median concentration was 84 mg/kg, with a range 61-202 mg/kg) (Tang *et al.*, 2000).

5.1.2.2 Refinery Chain of Commerce

Ethylbenzene is a common constituent of petroleum and coal tar (ATSDR, 1999). Automotive and aviation fuels contain ethylbenzene, including gasoline which typically contains about 2% ethylbenzene by weight (ATSDR, 1999). Ethylbenzene was found to be present as a constituent of solvents or contained in pesticides and as a component of commercial xylene (“mixed xylenes” used to make varnishes, paints, and lacquers), which may contain 6% to 15% ethylbenzene by volume (ATSDR, 1995). Ethylbenzene may be found in some consumer products, including inks and glues, and has been found to be a constituent of asphalt and of naphtha (ATSDR, 1999).

5.1.2.3 Other Uses

An additional source of exposure to ethylbenzene is tobacco products (ATSDR, 1995). Both smokers and non-smokers may be exposed to ethylbenzene released during the burning of tobacco. Ethylbenzene is also formed during the incomplete combustion of natural materials making it a component of forest fires (WHO, 1996).

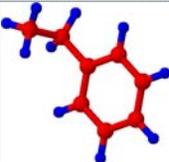
Ethylbenzene has also been used in the manufacture of acetophenone, cellulose acetate, diethylbenzene, ethyl anthraquinone, ethylbenzene sulfonic acids, propylene oxide, and *a*-methylbenzyl alcohol (HSDB, 2004).

5.2 Environmental Fate and Transport

5.2.1 Physical and Chemical Properties of Ethylbenzene

Ethylbenzene is a colorless, combustible liquid with a sweet, gasoline-like odor. Its major physical and chemical properties are summarized in Table 5-4. Additional information is available in the OECD Draft SIDS SIAR (OECD, 2005; included as Appendix A).

Table 5-4 Summary of Physicochemical Properties of Ethylbenzene^a

Property	Value
CAS number	100-41-4
Common synonyms	aethylbenzol, EB, ethylbenzol etilbenzene, etylobenzen, NCI-C56393 phenylethane, UN 1175
Empirical formula	C ₈ H ₁₀
Structural formula	(C ₆ H ₅)CH ₂ CH ₃
Structure	
Appearance	colorless, aromatic odor
Molecular weight	106.17 g/mol
Melting point	-95.°C
Boiling point	136.25° C
Flash point	21°C (70°F) (ATSDR, 1999)
Autoignition	432°C (810°F) (ATSDR, 1999)
Explosive limits	0.8 - 6.7 vol% in air (ATSDR, 1999)
Density	0.867 gm/mL @ 20°C 0.866 gm/mL @ 25°C (ATSDR, 1999)
Viscosity	0.64 cP @ 25°C (ATSDR, 1999)
Vapor pressure	9.53 mmHg @ 25°C 7 mmHg @ 20°C (ATSDR, 1999) 10 mmHg @ 25.9°C (ATSDR, 1999) 12 mmHg @30°C (ATSDR, 1999) 100 mmHg @ 74.1°C (ATSDR, 1999)
Solubility	140 mg/L @ 15°C 152 mg/L @ 20°C 169 mg/L @ 25°C 197 mg/L @ 0°C (ATSDR, 1999) 160 - 208 mg/L @ 25°C (ATSDR, 1999)
Octanol-Water Partition Coefficient (log K _{ow})	3.13 – 3.15 3.2 (Yaffe <i>et al.</i> , 2002)
Organic Carbon Partition Coefficient (log K _{oc})	2.6 (EPA, 2002a)
Henry's law constant	798.1 pa·m ³ /mol 6.6×10 ⁻³ - 8.7×10 ⁻³ atm·m ³ /mol@ 20°C (ATSDR, 1999) 7.88×10 ⁻³ - 8.43×10 ⁻³ atm·m ³ /mol@ 25°C (ATSDR, 1999)
Odor/Taste threshold	0.029 – 0.14 mg/liter in water (ATSDR, 1999) 0.09 ppm in air (low) (NOAA, 2004) 0.6 ppm in air (high) (NOAA, 2004) 2.3 ppm in air (OSHA, 2004)
Conversion Factors	1 mg/m ³ = 0.23 ppm 1 ppm = 4.35 mg/m ³

^a Source: OECD (2005), unless otherwise indicated.

5.2.2 Environmental Partitioning and Degradation

The fate and transport behavior of ethylbenzene in environmental media is briefly reviewed in the following sections.

5.2.2.1 Fugacity Modeling

The SIAR (OECD, 2005; Appendix A) for ethylbenzene presented the results of Mackay Level I and Level III fugacity multimedia modeling performed by Dow (2000). Primary input values for Level I and Level III modeling were: log K_{ow} , 3.15; water solubility, 169 g/m³; vapor pressure, 1,270 hPa; melting point, -95°C; Henry's law constant, 798.1 pa.m³/mol. Using the default emission values of 10,000 kg/yr and assuming equal distribution to all compartments, Level I modeling indicated that the majority of ethylbenzene will partition to the air compartment (98.6%) with only 0.6% to the water, 0.8% to the soil, and 0.02% to the sediment compartments. The high proportion in the air phase reflects the relatively high Henry's law constant of ethylbenzene. For the Mackay Level III (Equilibrium Criterion) Model, 2002 Toxics Release Inventory (TRI) data (see Table 5-10) and the information in Table 5-5 were used to determine appropriate emission rates of ethylbenzene to each individual compartment.

Table 5-5 Emission Input Values for Mackay Level III Fugacity Modeling ^a

Emission Rate	Kg/h (%)
to Air	333.8 (99.7)
to Water	0.52 (0.15)
to Soil	0.52 (0.15)
to Sediment	0 (0)

^a Source: 2002 TRI release data (see Table 5-10)

Results of the Mackay Level III Model are presented in Table 5-6 and Figure 5-3 (the printout is included as Appendix B). The Level III model calculation constrained the chemical of interest to steady state concentrations in each media. The chemical of interest is released into the individual compartments and can degrade within compartments. These results clearly show that the ultimate partitioning of ethylbenzene in the environment is expected to be in air, with minor partitioning to other compartments.

Table 5-6 Results of Mackay Level III Fugacity Modeling ^a

Media	Amount	Reaction Half-Life (h)
Bulk Air	98.5%	47
Bulk Water	0.67%	156
Bulk Soil	0.83%	156
Bulk Sediment	<0.01%	2,784

^a Source: Canadian Environmental Modelling Centre (2002)

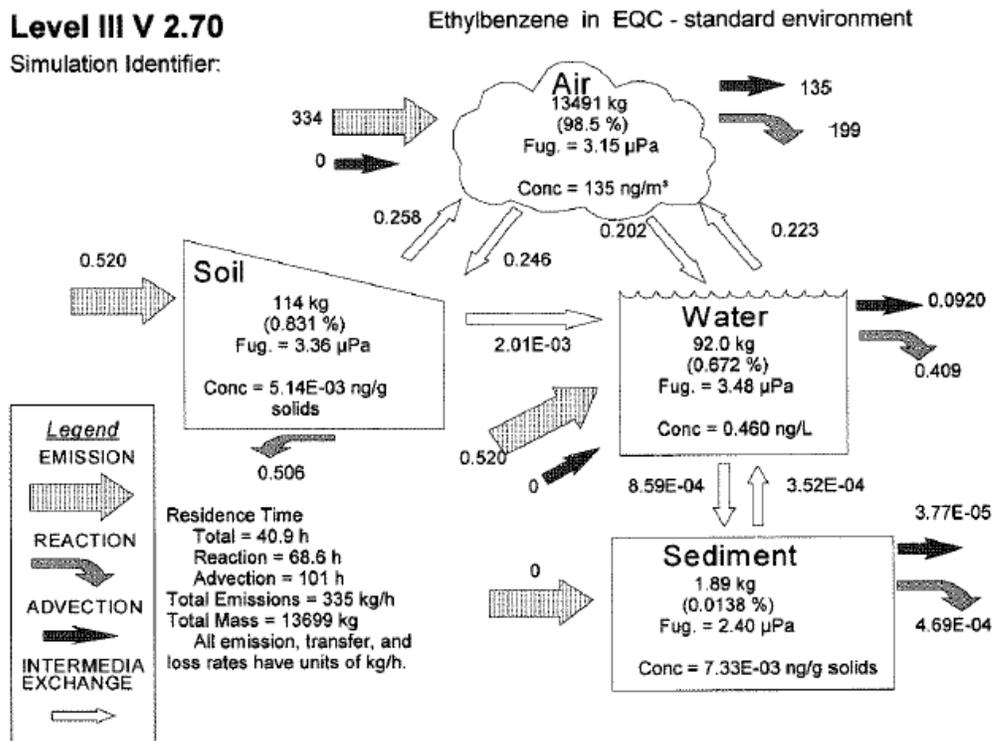


Figure 5-3. Mackay Level III Modeling Results: Ethylbenzene in Environmental Quality Control (EQC) Standard Environment (Level III v. 2.70)

5.2.2.2 Air

As indicated by the fugacity modeling, ethylbenzene is expected to partition primarily to the atmosphere, where it is expected to exist predominantly in the vapor phase until it is removed through physical processes. These processes include partitioning into clouds or rainwater, or interaction caused by the sun's energy (photo-oxidation) via reaction with hydroxyl radicals, nitrate (NO₃) radicals, atomic oxygen, ozone, and toluene. Ethylbenzene is not subject to direct photolysis, but may photochemically degrade by reaction with hydroxyl radicals (half-life 0.5 to 2 days) and partially return to earth in rain. Ethylbenzene is one of several chemicals believed to contribute to ozone formation that are monitored through EPA's Photochemical Assessment Monitoring Stations (PAMS). Oxidation by-products from the reaction with hydroxyl radicals and nitrogen oxides include ethylphenols, benzaldehyde, acetophenone, and *m*- and *p*-nitroethylbenzene (Hoshino *et al.*, 1978, cited in ATSDR [1999]). An atmospheric half-life of 2.7 days was estimated using the Atmospheric Oxidation Program (SRC, 1995, cited in ATSDR [1999]). Degradation occurs faster in summer months and under photochemical smog conditions (ATSDR, 1999).

5.2.2.3 Surface Water

Ethylbenzene is a water soluble aromatic hydrocarbon (ATSDR, 1999). When dissolved in surface water, soil pore water or groundwater, ethylbenzene is expected to migrate into the available atmospheric compartment until its saturated vapor concentration is reached

(ATSDR, 1999). Ethylbenzene is expected to biodegrade in surface water under either aerobic or anaerobic conditions with aerobic degradation being the more favorable (ATSDR, 1999). The biodegradation pathways shown in Figure 5-4 were listed in the University of Minnesota Biocatalysis/Biodegradation Database.

Under aerobic conditions, ethylbenzene degradation involved oxygenase reactions. Aerobic degradation can proceed in either of two primary pathways: (1) *Pseudomonas sp.* (strain NCIB 10643) initiated by a dioxygenation of the aromatic ring, leading to an extradiol ring cleavage and, (2) naphthalene dioxygenase that was capable of aerobically degrading ethylbenzene to styrene and/or 2-hydroxyacetophenone. Anaerobic degradation of ethylbenzene could be initiated by its dehydrogenation to 1-phenyl ethanol, and subsequent conversion to benzoate (Figure 5-4).

Photolytic transformations may also take place in surface waters in the presence of naturally occurring humic materials (sensitized photolysis). Half-life in water has been estimated to be 13 days in winter, 20 days in spring, and 0.1 days in summer (ATSDR, 1999).

5.2.2.4 Groundwater

Ethylbenzene was found to be mobile in sand and gravel aquifers and other aquifers that contained little solid-phase organic matter (Ptacek *et al.*, 1984, cited in ATSDR [1999]). When ethylbenzene was part of a complex mixture of hydrocarbons associated with a petroleum spill or leak, the proportion of ethylbenzene that was estimated to be bound to soil versus the amount that was estimated to migrate to groundwater depended primarily on site- and mixture-specific characteristics. Biodegradation of ethylbenzene in groundwater can occur via both aerobic and anaerobic processes; mechanisms and rates were dependent upon site-specific conditions (Figure 5-4) (WHO, 1996; ATSDR, 1999).

5.2.2.5 Soil

Based on its K_{oc} value and using the classification scheme of Swann *et al.* (1983) (cited in ATSDR [1999]), ethylbenzene was classified as having moderate mobility in soils. Sorption and retardation by soil organic carbon content is expected to occur to a moderate extent, but particularly in soils with low organic carbon content, ethylbenzene will tend to leach into groundwater (WHO, 1996; ATSDR, 1999).

In soil and sediment, as in surface water, ethylbenzene can be biodegraded under both aerobic and anaerobic conditions; however, aerobic biodegradation occurred more rapidly (Figure 5-4). Aerobic biodegradation of ethylbenzene has been reported in the presence of several soil microbes. Anaerobic degradation of ethylbenzene from soil and sediment was much slower than under aerobic conditions. The kinetics of biodegradation appear to be site-specific, and depended upon a number of factors, such as the type and population of microbes present, the environmental conditions, the concentration of ethylbenzene, and the presence of other compounds that may act as a substrate. Biodegradation in soil will also compete with more rapid migration processes, such as volatilization and infiltration to groundwater (ATSDR, 1999).

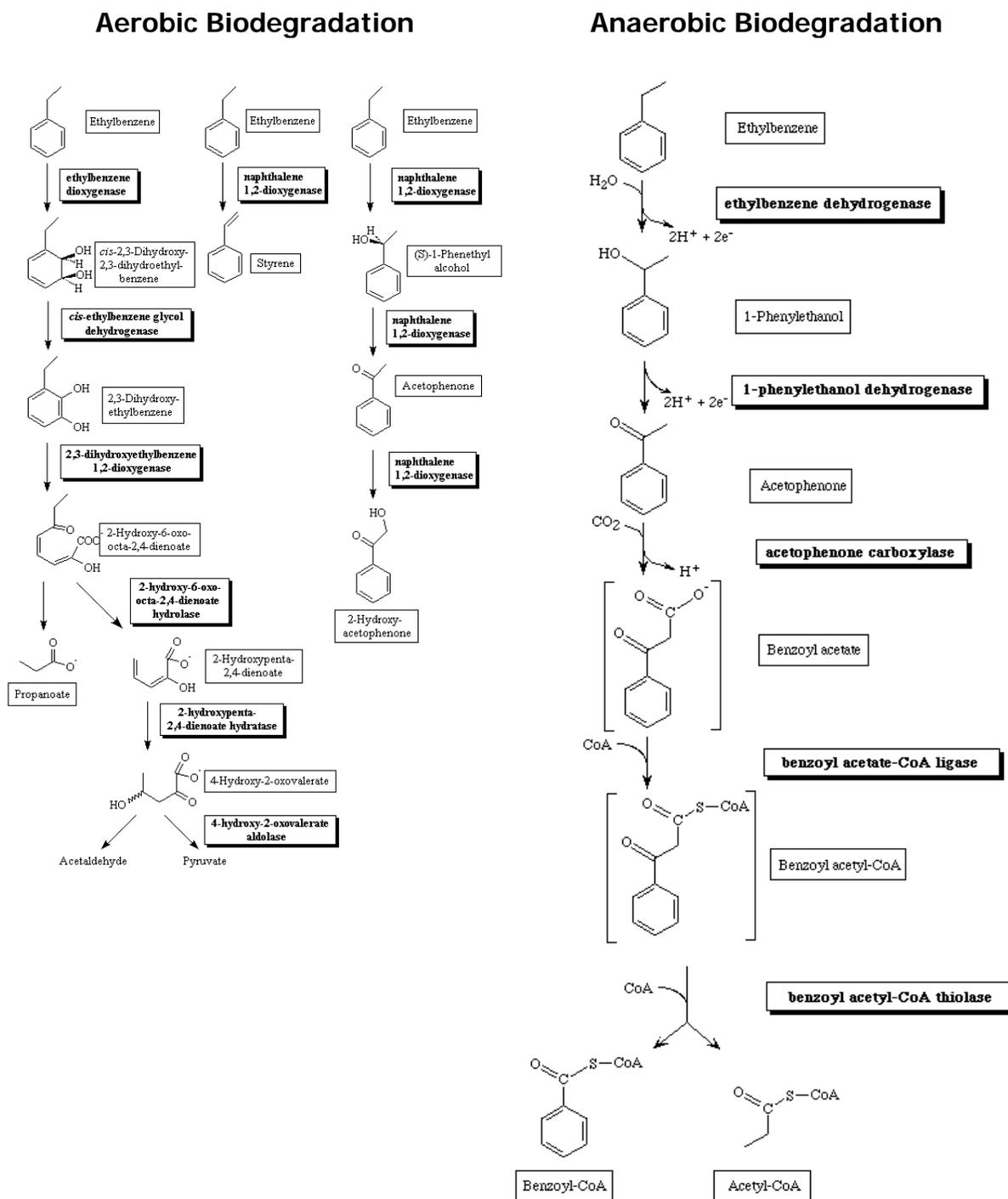


Figure 5-4. Aerobic and Anaerobic Biodegradation Pathways for Ethylbenzene (Source: University of Minnesota [2004])

5.2.2.6 Sediment

The physicochemical properties of ethylbenzene indicate that it would not sorb appreciably to sediment (WHO, 1996; ATSDR, 1999).

5.2.3 Bioaccumulation

Due to its physicochemical properties, ethylbenzene is not expected to significantly bioaccumulate in terrestrial food chains. Based on $\log K_{ow}$, a theoretical bioconcentration factor (BCF) of approximately 100 was calculated (OECD, 2005); however, bioconcentration factors of only 1.1 to 15 were measured in four species of fish and two studies in clams (Ogata *et al.*, 1984; Nunes and Benville, 1979). No aquatic food chain magnification was predicted from the model calculations and empirical observations by Thomann (1989).

5.2.4 Summary

As a low molecular weight volatile organic compound (VOC), ethylbenzene is mobile in all environmental media, with a strong tendency to migrate to the atmosphere regardless of the mode of release. With low affinity for soils and sediments, it does not accumulate in soil or sediment but can leach to groundwater. It is not persistent in any medium, being degraded primarily by photo-oxidation and biodegradation. However, because the atmospheric photo-oxidation of ethylbenzene may contribute to ozone formation, it is a PAMS target compound.

Although the $\log K_{ow}$ of approximately 3 indicates some potential for bioaccumulation, available evidence showed that actual ethylbenzene bioconcentration factors for fish and mollusks were low, perhaps due to rapid elimination.

The physicochemical characteristics and behavior of ethylbenzene in the environment indicate that the most likely route of human exposure is inhalation.

5.3 Environmental Releases of Ethylbenzene

Ethylbenzene is continuously released to the environment from natural sources and processes, industrial processes and products, and human activities. These sources can be generally categorized as stationary (*e.g.*, prescribed and uncontrolled burning, residential burning, incinerators, factories, refineries, power plants), mobile (*e.g.*, combustion engines in lawnmowers, motorboats, cars, trucks, buses, motorcycles, off-road vehicles and non-road machines, trains, airplanes), and personal (*e.g.*, use of consumer products containing mixed xylenes and ethylbenzene in tobacco smoke).

In the absence of a single comprehensive compilation of ethylbenzene emissions from all sources, the two major publicly available databases, the National Emissions Inventory (NEI) and TRI, were queried to develop as complete a picture as possible of the absolute and relative magnitude of ethylbenzene releases from recognized sources. In addition, an attempt was made to quantify emissions from cigarette smoking, an important source of ethylbenzene in indoor air (Section 5.3.3).

A primary focus of this Tier 1 exposure assessment is the delineation of the contribution of the ethylbenzene/styrene chain of commerce sources to the total ethylbenzene exposure to children and prospective parents. Therefore, particular emphasis was placed on quantifying

the relative contribution of ethylbenzene emissions from facilities directly involved in the ethylbenzene/styrene chain of commerce.

5.3.1 Total Ethylbenzene Emissions (NEI Database)

The 1990 Clean Air Act (CAA) requires EPA to identify sources, quantify emissions by source category, develop regulations for each source category, and assess public health and environmental impacts of 188 hazardous air pollutants (HAPs), including ethylbenzene. Developed to address this mandate, the NEI is a comprehensive inventory covering all anthropogenic sources of criteria pollutants and HAPs for all areas of the United States. As such, it provides an overall picture of ethylbenzene emissions.

The NEI includes three classes of HAP emission sources:

- **Point sources:** stationary (point) sources that emit or have the potential to emit at least ten tons per year or more of any listed HAP, or 25 tons per year or more of a combination of listed HAPs;
- **Area sources:** stationary sources that emit or have the potential to emit less than ten tons per year of a single HAP, and less than 25 tons per year of all HAPs combined, including wildfires and prescribed burning;
- **Mobile sources:** on-road vehicles, non-road diesel engines, off-road vehicles, aircraft, locomotives, and commercial marine vessels.

The contributions from various source categories to ethylbenzene emissions were retrieved using the 1999 NEI database¹ (Table 5-7). Point and area (non-point) summaries were available in the database. For mobile source emissions, each state contribution was summed and the totals were incorporated into a summary for purposes of developing the overall ethylbenzene contribution. Emissions from point sources are presented in Appendix C.

Table 5-7. Annual Average Ethylbenzene from the 1999 National Emissions Inventory Database^a

Category	Annual Average Emission (ton/yr)
Point (major)	10,600
Area (Non-Point)	29,231
Mobile (On-road)	70,075
Mobile (non-road)	44,137
Total	154,043

^a Source: EPA, 1999.

As indicated in Table 5-7, a total of 154,043 tons (308 million pounds) of ethylbenzene was emitted from the three major source categories in 1999. Of this total, mobile sources (on-road and off-road source combined) contributed the majority (228 million pounds, 74%). Area source contributions totaled 19% (58 million pounds), and the smallest contribution

¹ At the time this submittal was completed, the 2002 NEI database had not been finalized. While a draft version of this database was available, numerous reported errors prohibited its use.

came from major point sources (21 million pounds; 7%) (Figure 5-5). The major sources contributing to the area emission source total of 29,231 ton/yr were asphalt applications (47%), surface coatings (37%), and gasoline distribution (7%). None of these sources are considered part of the ethylbenzene/styrene chain of commerce.

The total ethylbenzene emissions for each individual Standard Industrial Classification (SIC) codes are presented in Appendix C with the data sorted in descending order of total emissions. In Appendix C, the 10 SIC categories that contributed the most to total ethylbenzene emissions include: Paper and Allied Products, Pulp Mills; Transportation Equipment, Motor Vehicles and Equipment, Motor Vehicles and Car Bodies; Petroleum and Coal Products, Petroleum Refining; Paper and Allied Products, Paper Mills; Paper and Allied Products, Paperboard Mills; Unlisted; Electric, Gas, and Sanitary Services, Sanitary Services, Refuse Systems; Chemicals and Allied Products, Industrial Organic Chemicals (the first SIC code in the major group 28 or 30 range); Furniture and Fixtures, Household Furniture; Wood Household Furniture; and Transportation Equipment, Motor Vehicles and Equipment, Motor vehicles parts and accessories.

The NEI data demonstrated that major industrial facilities were a relatively minor source of ethylbenzene emissions in the U.S., less than 7% (Figure 5-5). Moreover, industries under SIC codes 28 and 30, which include facilities directly involved in the ethylbenzene/styrene chain of commerce, contributed 931.6 tons per year (8.8% to the total point-source emissions and only 0.6% of the total ethylbenzene annual average emission of 154,043 tons/yr) (Figure 5-5) reported in the 1999 NEI database. This is not surprising since the process for making ethylbenzene and styrene takes place in a closed system, minimizing the potential for release and for worker exposure (OECD, 2005).

5.3.2 Major Industrial Ethylbenzene Emissions (TRI Database)

The TRI is a publicly available EPA database that contains information about releases and other waste management activities reported annually by certain covered industry groups, as well as federal facilities for over 650 chemicals, including ethylbenzene (<http://www.epa.gov/tri/>). TRI reporting is required only for facilities that: (1) have ten or more full-time employees or the equivalent; (2) are included in specified industrial sectors (Table 5-8); and, (3) exceed any of the following reporting thresholds for manufacturing, processing, or otherwise using a TRI chemical:

- Manufactured (including imported) more than 25,000 pounds per year; or
- Processed more than 25,000 pounds per year; or
- Otherwise used more than 10,000 pounds per year.

TRI data are included in the “major sources” category in the NEI database. Inclusion of information on specific emission routes in the TRI database allows more detailed analysis of the contributions of particular sources. The currently available TRI database has been updated through 2002 (www.epa.gov/triexplorer). Total and industry-specific releases were examined and compared in terms of quantities and routes of emission.

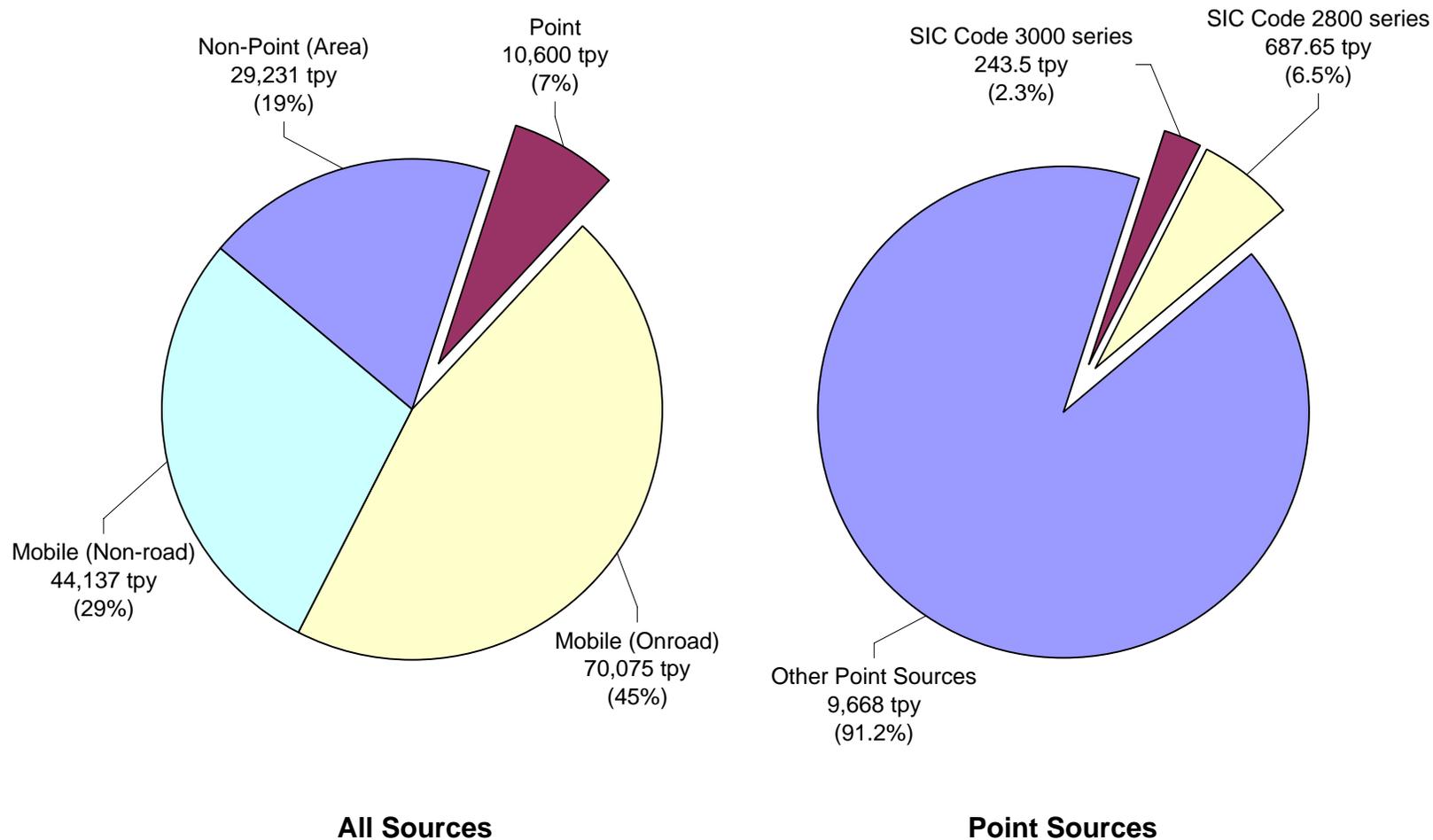


Figure 5-5. Relative Contributions of Different Sources to Total Ethylbenzene Emissions, 1999 (tons per year, % of Total) (data from NEI 1999 database)

5.3.2.1 Total Industrial Emissions

Trends in total on-site and off-site releases of ethylbenzene for all TRI reporting facilities in the U.S. from 1988 through 2002 are presented in Table 5-9 and Figure 5-6. In 2002, 99% of total releases were on-site and 1% off-site (Table 5-9).

Trends in on-site releases to specific media are presented in Table 5-10 and Figure 5-7. As expected, the major release of ethylbenzene is atmospheric, with 86% or more of the releases in each year occurring to the air.

Table 5-8. Standard Industry Classification Codes of Industry Sectors Subject to Toxics Release Inventory Reporting ^a

20 Food
21 Tobacco
22 Textiles
23 Apparel
24 Lumber and Wood
25 Furniture
26 Paper
27 Printing and Publishing
28 Chemicals
29 Petroleum and Coal
30 Rubber and Plastics
31 Leather
32 Stone, Clay, and Glass
33 Primary Metal
34 Fabricated Metals
35 Machinery (excluding electrical)
36 Electrical and Electronic Equipment
37 Transportation Equipment
38 Instruments
39 Miscellaneous Manufacturing
10 Metal mining (except for SIC codes 1011, 1081, and 1094)
12 Coal mining (except for 1241 and extraction activities)
Electrical utilities that combust coal and/or oil (SIC codes 4911, 4931, and 4939)
4953 RCRA Subtitle C hazardous waste treatment and disposal facilities
5169 Chemicals and allied products wholesale distributors
5171 Petroleum bulk plants and terminals
7389 Solvent recovery services
^a Source: EPA (2004b).

In terms of the geographical distribution of ethylbenzene releasing facilities, the top five states are Texas, Louisiana, Michigan, Illinois, and Iowa, with total releases ranging from 1.7 to 16 million pounds in 2002. The bottom five states are Arizona, Idaho, Maine, Nevada,

and Vermont, with total releases ranging from 513 to 5,890 pounds in 2002. A county detail map (Figure 5-8) shows that release areas tend to be quite localized in all states.

Table 5-9. Trends in On- and Off-Site Disposal and Releases of Ethylbenzene for Facilities in All Industries, 1988 – 2002 (Millions of Pounds)^a

Year	Total On-Site Disposal or Release	Total Off-Site Disposal or Release	Total Disposal or Release
1988	7.98	0.42	8.40
1989	10.25	0.61	10.87
1990	10.15	0.38	10.53
1991	9.47	0.20	9.67
1992	10.74	0.14	10.88
1993	10.86	0.13	10.99
1994	12.83	0.30	13.13
1995	10.83	0.17	11.00
1996	10.00	0.10	10.10
1997	9.74	0.08	9.82
1998	9.71	0.17	9.88
1999	9.77	0.36	10.13
2000	9.03	0.15	9.17
2001	7.24	0.39	7.63
2002	7.55	0.10	7.65

^a Source: EPA (2005c).

Table 5-10. Disposal and Releases of Ethylbenzene by Media (Millions of Pounds)^a

Year	Fugitive Air Emissions	Stack Air Emissions	Surface Water Discharges	Underground Injection	Release to Land
1988	3.21	4.51	0.02	0.07	0.18
1989	3.49	6.60	0.02	0.06	0.09
1990	3.25	6.61	0.01	0.21	0.06
1991	3.07	6.23	0.02	0.09	0.05
1992	3.33	6.91	0.02	0.19	0.29
1993	3.09	7.39	0.02	0.33	0.03
1994	3.31	8.81	0.01	0.63	0.05
1995	2.71	7.62	0.01	0.48	0.02
1996	2.46	7.14	0.01	0.34	0.06
1997	2.21	6.82	0.01	0.56	0.15
1998	2.26	6.45	0.01	0.76	0.21
1999	2.33	6.52	0.01	0.88	0.04
2000	1.96	6.44	0.02	0.56	0.05
2001	1.74	4.82	0.01	0.65	0.02
2002	1.66	4.91	0.01	0.96	0.01

^a Source: EPA (2005c).

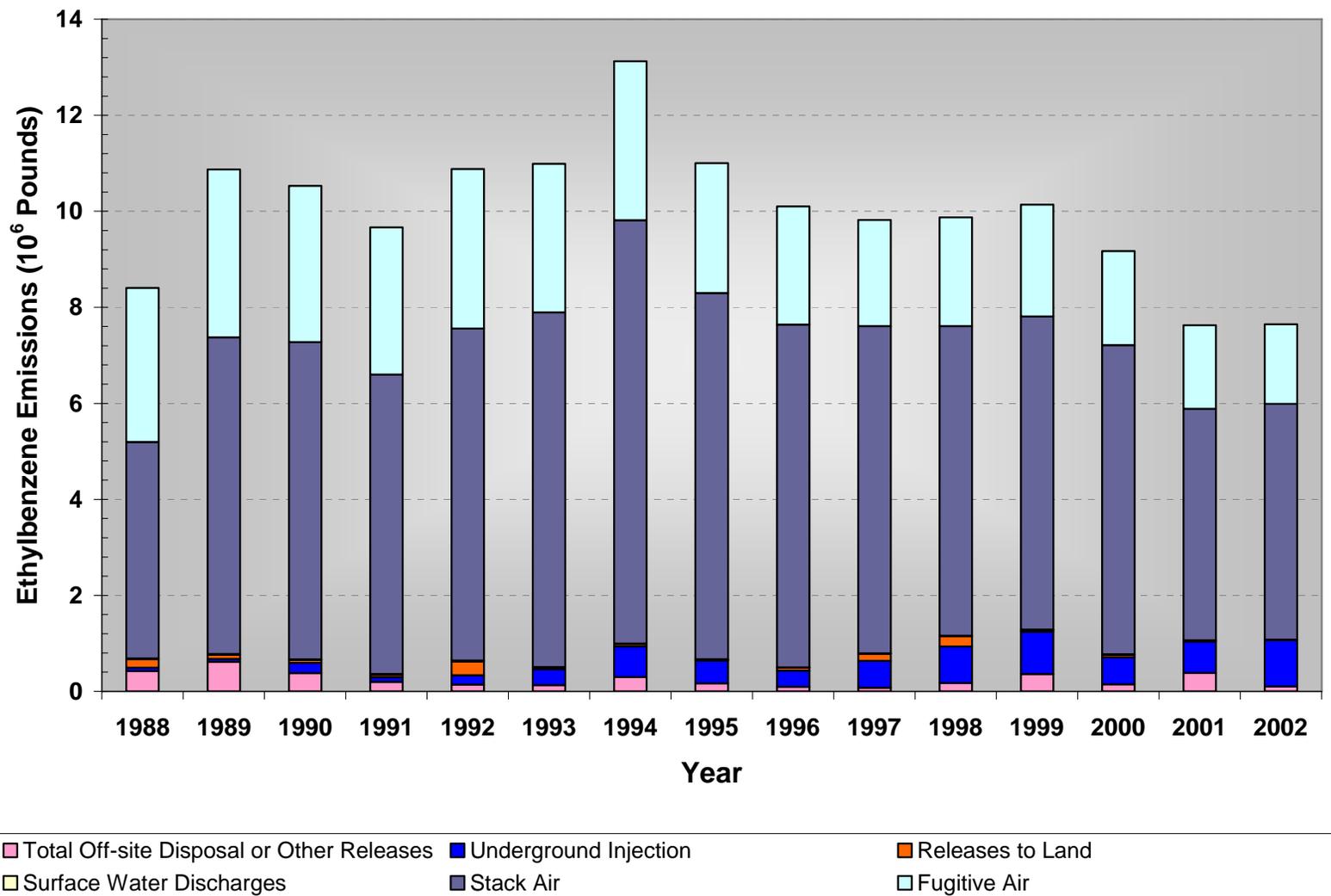


Figure 5-6. Trends in Total Ethylbenzene Releases to Air, Water, and Land (from TRI database)

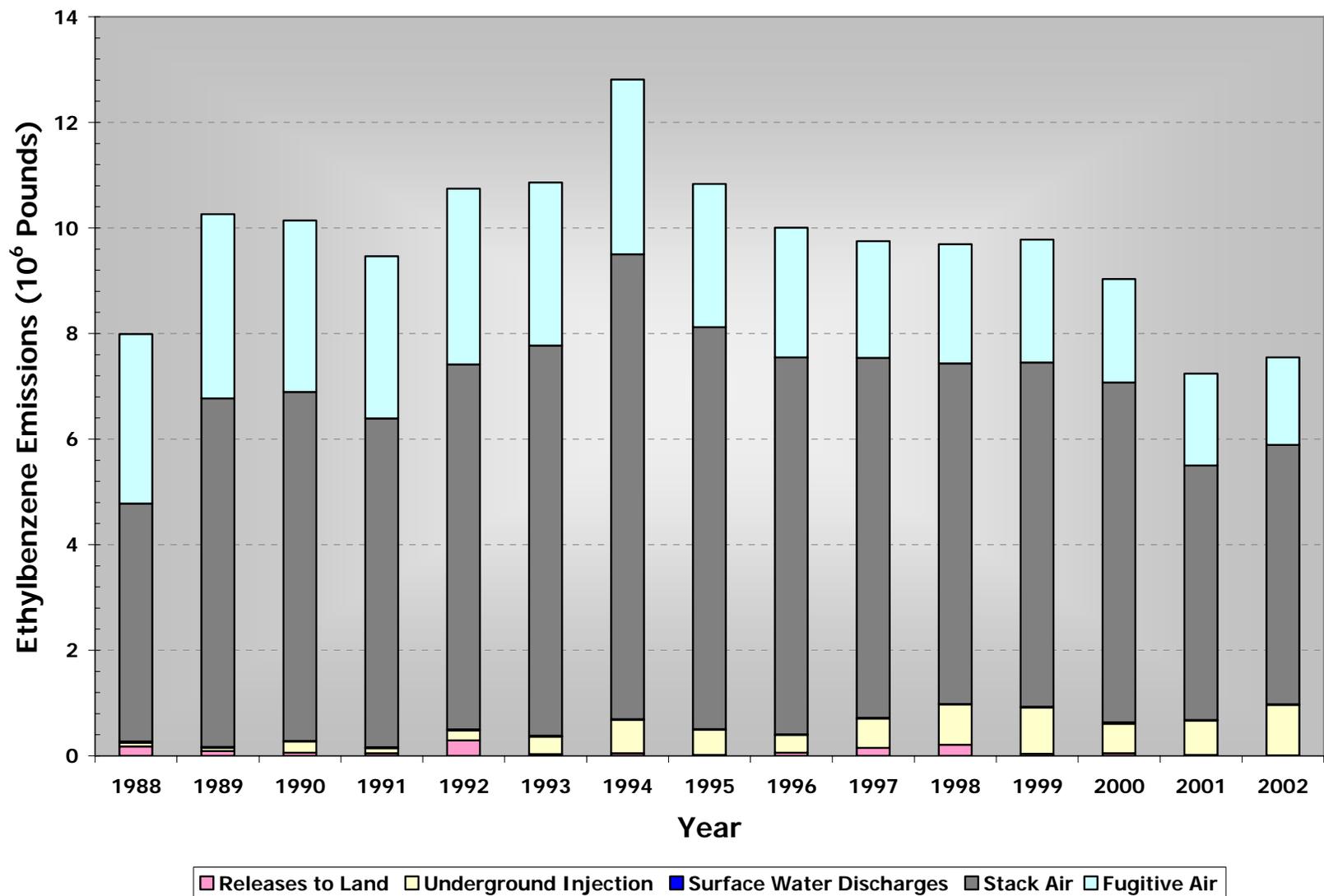


Figure 5-7. Trends in On-Site Ethylbenzene Releases to Air, Water, and Land (from TRI database)

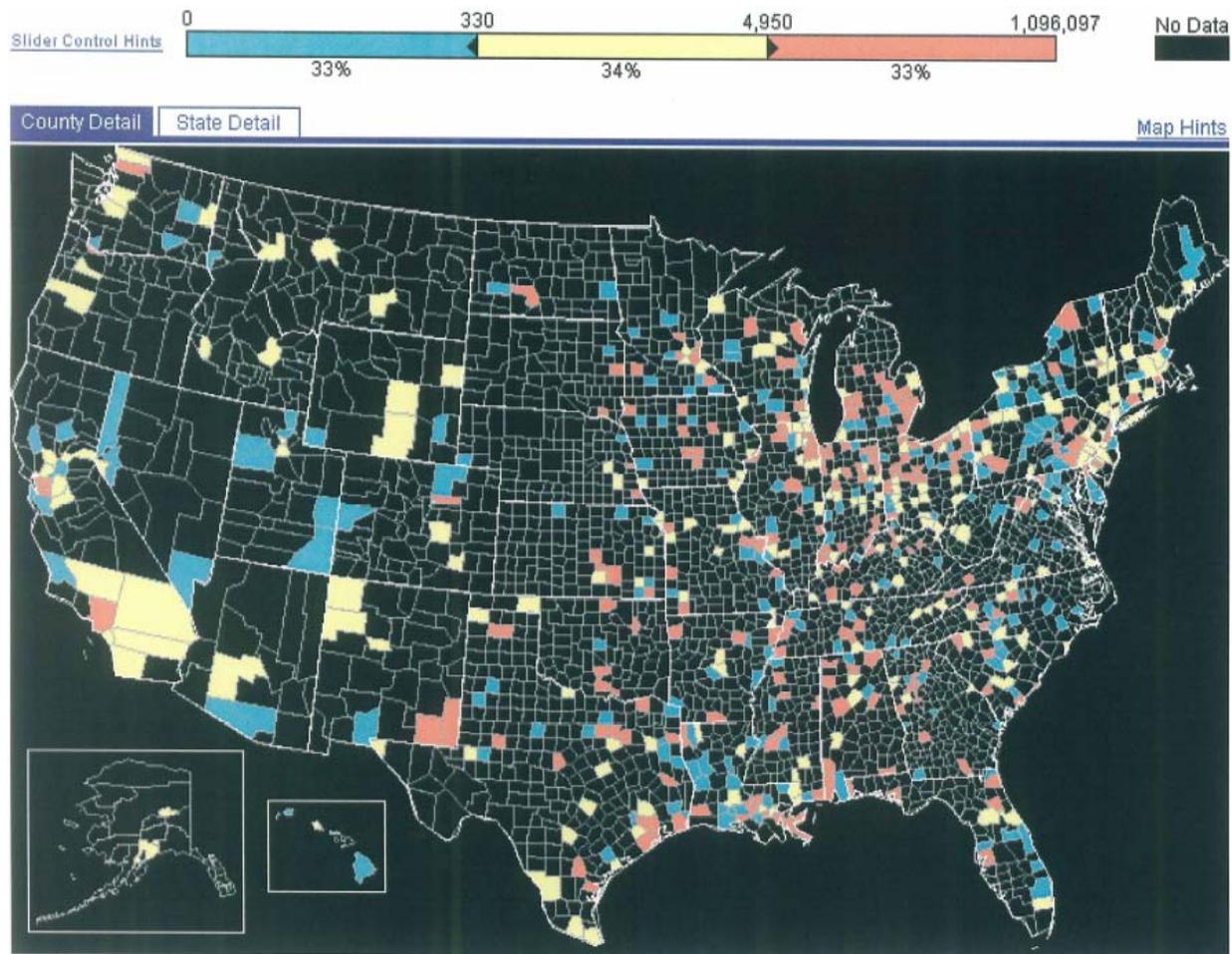


Figure 5-8. Map of Total On- and Off-Site Disposal or Other Releases of Ethylbenzene, 2002 (from TRI database)²

² Scale indicates pounds released.

5.3.2.2 Relative Industrial Contributions

In order to distinguish the relative contributions of specific sources, industries were sorted by two-digit SIC codes. As shown in Table 5-11, leading contributors to ethylbenzene releases or disposal in the TRI database were transportation equipment (SIC code 37), chemicals (SIC code 28), and petroleum (SIC code 29).

Table 5-11. Total Ethylbenzene Releases and Disposals by Industry Category, 2002^a

Industry	SIC Code	2002 Total Ethylbenzene Emissions (10 ⁶ Pounds)	Percent of Total
Transportation Equipment	37	2.56E+00	34%
Chemicals	28	1.83E+00	24%
Petroleum	29	8.96E-01	12%
Lumber	24	6.57E-01	9%
Fabricated Metals	34	4.52E-01	6%
Plastics	30	4.75E-01	6%
Primary Metals	33	1.82E-01	2%
Petroleum Bulk Terminals	5171	1.12E-01	1%
Furniture	25	9.83E-02	1%
Electrical Equip.	36	9.33E-02	1%
Machinery	35	7.59E-02	1%
RCRA/Solvent Recovery	4953/7389	4.71E-02	1%
Miscellaneous	39	4.66E-02	1%
Paper	26	4.19E-02	1%
Stone/Clay/Glass	32	2.30E-02	<1%
Measure/Photo.	38	7.54E-03	<1%
Chemical Wholesalers	5169	6.32E-03	<1%
No Reported Codes		3.79E-03	<1%
Printing	27	3.17E-03	<1%
Apparel	23	6.46E-04	<1%
Textiles	22	1.74E-04	<1%
Food	20	5.00E-06	<1%
Total		7.61E+00	

^a Source: EPA (2005c).

As indicated in Table 5-1, the ethylbenzene-producing facilities currently in the U.S. are located in Louisiana and Texas. Ethylbenzene releases or disposals from these facilities in 2002 (Appendix D) were identified and compared to total ethylbenzene releases or disposals and to releases or disposals from industries in the major SIC categories 28 and 30 in the TRI database (Table 5-1, Table 5-12, Figure 5-9). Total releases or disposals from the ethylbenzene-producing facilities in 2002 were 1.11 million pounds and comprised 14% of total releases or disposals reported in the TRI, while releases or disposals from facilities within the SIC categories 28 and 30 contributed 2.3 million pounds (30%). The major route of ethylbenzene release or disposal from all U.S. facilities was in air emissions (86%) (both point source and fugitive air emissions). The majority of ethylbenzene released or disposed

of by producers was injected into deep (Class I) wells (68.5%), a disposal method that involves negligible potential for human exposure (Figure 5-10). Air emissions from ethylbenzene producers totaled 0.34 million pounds in 2002, approximately 5% of total emissions to air of 6.57 million pounds from all U.S. facilities.

Table 5-12. Contribution of Ethylbenzene Producers to Total U.S. Industrial Disposal and Releases of Ethylbenzene, 2002^a

Release	Ethylbenzene Disposed or Released, 2002 (10 ⁶ Pounds)		
	All U.S. Facilities	Facilities in Major SIC Groups 28 and 30	Ethylbenzene Producers ^b
On-Site Disposal and Release			
Class I Wells	9.59E-01	7.60E-01	7.60E-01
RCRA Subtitle C Landfills	1.87E-03	1.81E-03	1.31E-03
Other On-Site Landfills	9.70E-04	9.55E-04	6.81E-04
Fugitive Air Emissions	1.66E+00	6.14E-01	1.71E-01
Point Source Air Emissions	4.91E+00	8.98E-01	1.70E-01
Surface Water Discharges	1.08E-02	3.50E-03	9.32E-04
Class II-V Wells	1.50E-05		
Land Treatment	4.43E-05		
Surface Impoundments	1.90E-05		
Other Land Disposal	5.31E-03	6.26E-04	
Total On-Site	7.55E+00	2.28E+00	1.10E+00
Off-Site Disposal and Release			
Underground Injection	5.23E-03	4.35E-03	
RCRA Subtitle C Landfills	5.04E-03	3.19E-03	1.92E-04
Other Landfills	1.04E-02	1.61E-03	7.73E-04
Other Storage Only	1.17E-02	8.35E-03	1.80E-03
Other Surface Impoundments	2.58E-04		
Other Land Treatment	2.74E-04	2.40E-04	
Other Land Disposal	2.14E-03	5.92E-04	
Other Off-Site Management	9.18E-03	6.84E-04	
Other Waste Broker	8.93E-03	1.28E-03	
Other Unknown	1.11E-02	4.31E-03	
Total Off-Site	6.42E-02	2.46E-02	2.77E-03
Grand Total	7.61E+00	2.30E+00	1.11E+00
^a Source: EPA (2005c).			
^b See Appendix D.			

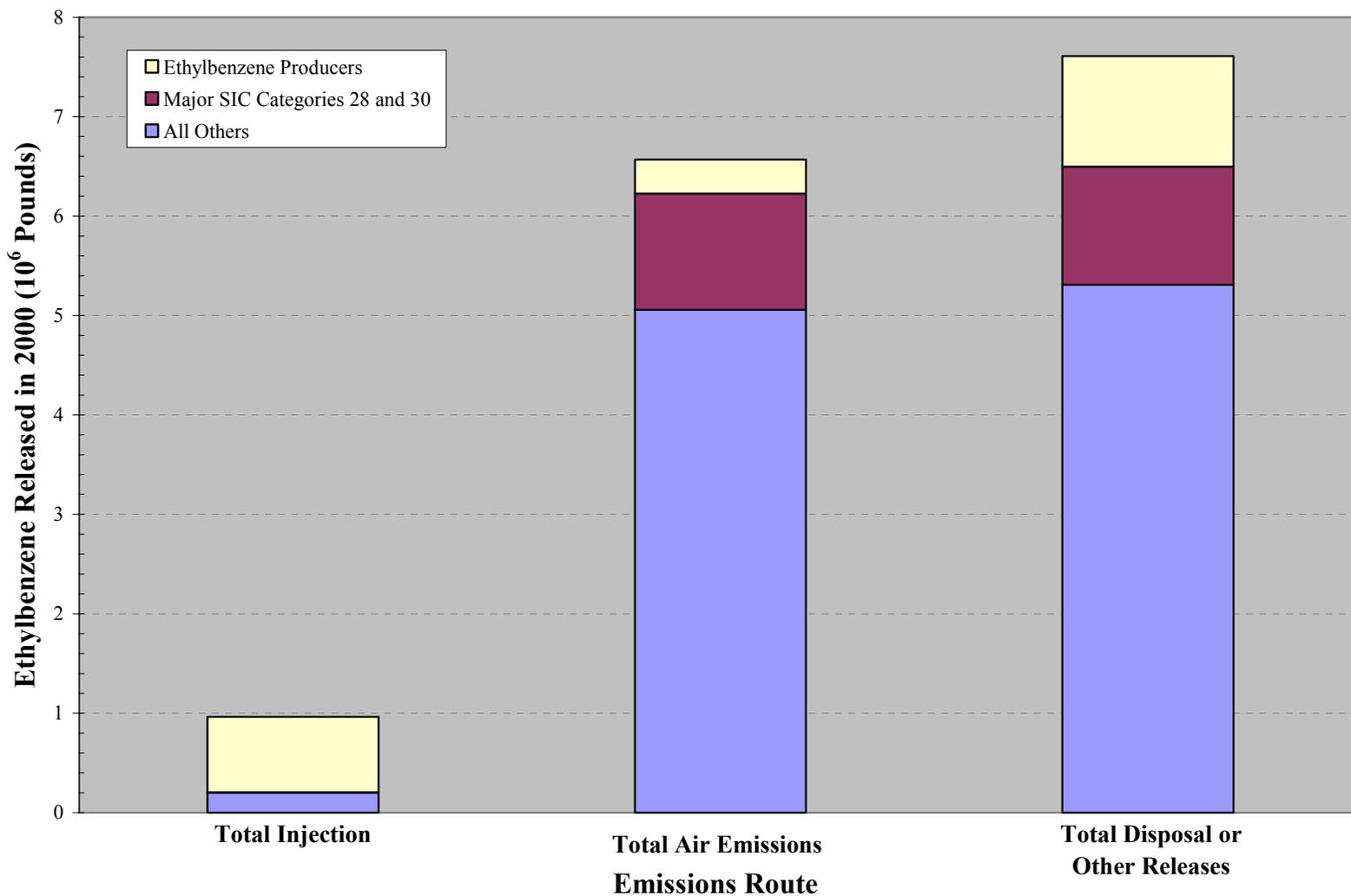


Figure 5-9. Major Routes of Ethylbenzene Emissions by Ethylbenzene Producers vs. All Other Facilities (from TRI database)

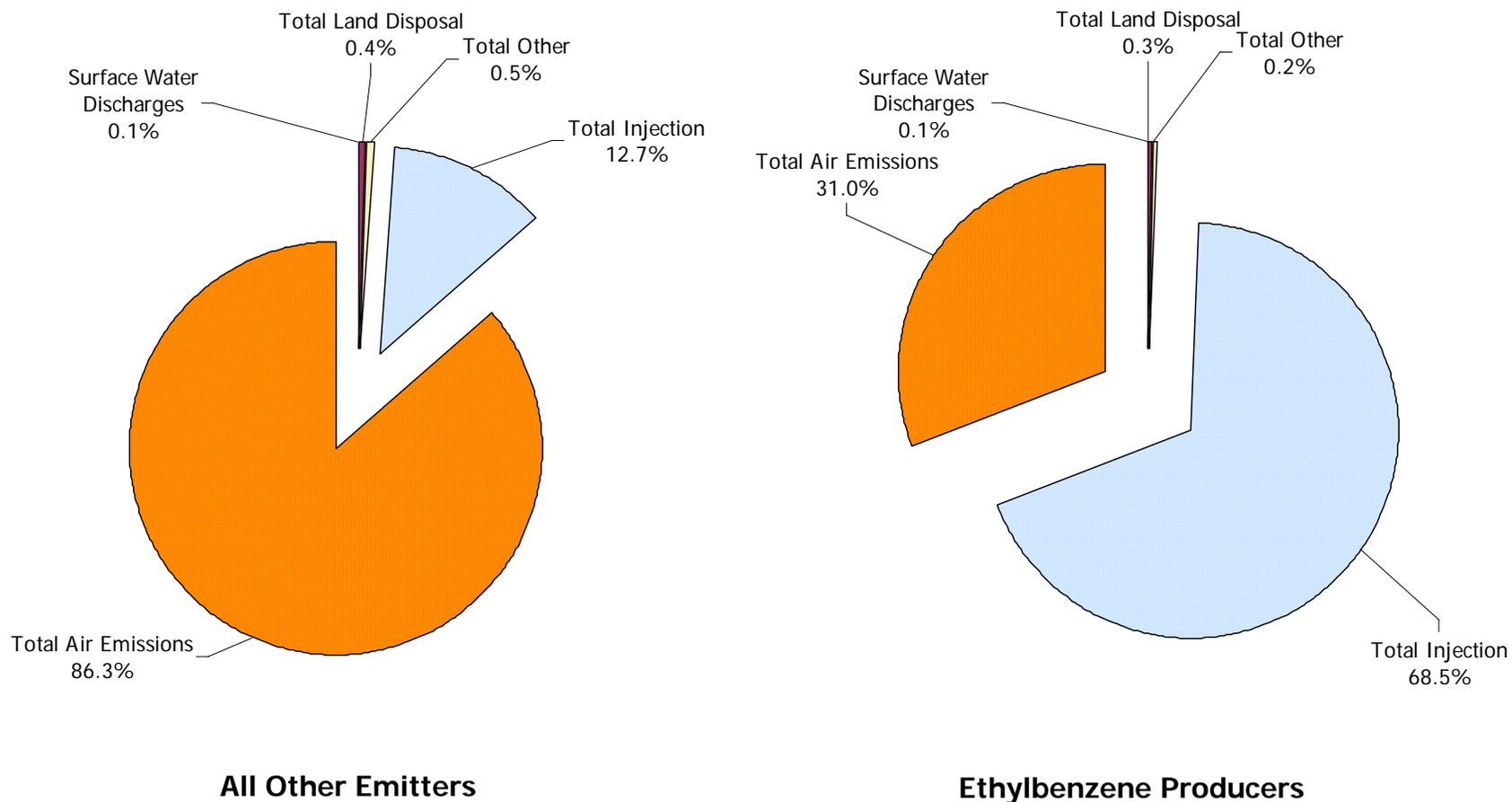


Figure 5-10. Comparison of Proportion of Ethylbenzene Emissions by Different Routes, Ethylbenzene Producers vs. All Industrial Sources (from TRI database)

5.3.3 Environmental Tobacco Smoke

A recognized source of ambient ethylbenzene not included in the NEI database is the combustion of tobacco products. Six commercial cigarette brands averaged 130 μg ethylbenzene per cigarette (CARB, 1997). According to the USDA (2004), 400 billion cigarettes were consumed in the United States in 2003. Based on these values, the annual estimated emission of ethylbenzene due to cigarette smoke would be about 110,000 pounds as calculated in Equation (5-1).

$$\begin{aligned} \text{Ethylbenzene emission} \left[\frac{\text{lb}}{\text{yr}} \right] &= 130 \frac{\mu\text{g}}{\text{cigarette}} \times 4\text{E}+11 \frac{\text{cigarettes}}{\text{year}} \times 10^{-9} \frac{\text{kg}}{\mu\text{g}} \times 2.2 \frac{\text{lb}}{\text{kg}} \quad (5-1) \\ &= 1.1\text{E}+05 \end{aligned}$$

5.3.4 Summary

Releases associated with the ethylbenzene/styrene chain of commerce can occur from sites of ethylbenzene/styrene production, from the processing of styrene monomer into polymers, and from the further processing of the styrenic polymers to make products. As the plastics produced can contain residual ethylbenzene, releases are also possible during the lifetime and following disposal of the articles. However, available data suggest that such releases are very small (see Section 6.3.1.3).

Much more quantitatively significant are the refinery chain of commerce and other sources of ethylbenzene emissions released to the environment, such as releases from petroleum refining, combustion processes, cigarettes, and automobile emissions. Data from the two publicly available databases, NEI and TRI, both clearly support the conclusion that industries directly involved in the ethylbenzene/styrene chain of commerce are responsible for only a very small proportion of ethylbenzene emissions in the U.S. The two databases yielded similar estimates of total air emissions from facilities involved in the ethylbenzene chain of commerce, assuming these facilities consisted of the major SIC categories 28 and 30.

Data from the TRI database indicate that the major route of industrial ethylbenzene emission is release to air. In contrast, the bulk of “emissions” from the major ethylbenzene producers identified in Table 5-1 is deep well injection, with little or no possibility for human exposure. The total air emissions from the ethylbenzene/styrene chain of commerce (considered as the major SIC code categories 28 and 30) was 1.5 million pounds, while the emissions from the ethylbenzene producers in 2002 (see Table 5-1) was 0.34 million pounds, 5% of the total major industrial air emissions. This producers emission result is of a similar order of magnitude as that estimated from cigarette smoking (0.11 million pounds).

The broader NEI database showed that the majority of ethylbenzene emissions were derived from mobile sources, not from major industrial point sources. All emissions from major point sources were relatively small, around 7% of the total ethylbenzene emitted. Those from the major SIC code categories 28 and 30, which include chain of commerce facilities, comprise 1.9 million pounds, 8.8% of emissions from major point sources and around 0.6% of the total from all sources. Based on these results, it is reasonable to conclude that facilities

directly involved in the ethylbenzene/styrene chain of commerce are currently responsible for only a very small fraction of total ethylbenzene emissions in the U.S.

The data reviewed in this section support the conclusion that contact directly connected with the ethylbenzene/styrene chain of commerce, including exposures from production and use of ethylbenzene as a neat compound, is a minor contributor to exposure. As such, exposure to children and prospective parents not employed as ethylbenzene production workers is likely to be dominated by refinery chain of commerce sources, such as automobile exhaust and consumer products containing mixed xylenes, or other sources, such as tobacco smoke.

6.0 EXPOSURE ASSESSMENT

6.1 Ethylbenzene Levels in Human Tissues

Ethylbenzene and other VOCs typically have very short half-lives in humans, but their widespread presence in the environment may be a source of continued exposure to most members of the general public. Ethylbenzene and other common VOCs (and their metabolites) have been found in human tissues, including breath, blood, urine, and milk. A number of studies (discussed in the following sections) have been conducted to quantify levels of ethylbenzene and other common VOCs in breath and blood from adult members of the general public, and one recent study focused on children (Sexton *et al.*, 2005). The results of these studies provide insight into not only typical ethylbenzene levels but also important sources of exposure for the general public. In the absence of monitoring data for ethylbenzene in human milk, a physiologically based pharmacokinetic (PBPK) model was used to estimate concentrations (see Section 6.1.3).

6.1.1 Exhaled Breath

Between 1980 and 1984, the EPA carried out a study of human exposure to VOCs called the TEAM study (see Section 6.2.2). In this study, 26 chemicals, including ethylbenzene, were measured in the personal air, drinking water, and exhaled breath of 523 adults representing residents of five cities (Bayonne and Elizabeth, NJ, and Antioch, Pittsburg, and Los Angeles, CA). Ethylbenzene levels (as well as those of benzene, styrene, xylenes, and several other compounds) were two to seven times (and statistically significantly) higher in the exhaled breath of smokers than non-smokers (Table 6-1), with a strong exposure-dose relationship between number of cigarettes smoked per day and breath VOC levels (Wallace *et al.*, 1986, 1987). Levels were also increased in non-smokers exposed to heavy smokers at work (Wallace *et al.*, 1986, 1987). These results demonstrate the importance of tobacco smoking as a source of ethylbenzene exposure.

Table 6-1. Breath Concentrations ($\mu\text{g}/\text{m}^3$) of Ethylbenzene in Smokers vs. Non-Smokers in the TEAM Study ^a

Location (Season)	Smoker	Non-Smoker
New Jersey (fall)	3.9	2.0
New Jersey (summer)	2.0	0.5
New Jersey (winter)	2.3	1.1
Los Angeles (winter)	2.4	0.6
Los Angeles (spring)	3.2	0.5
Antioch/Pittsburg, CA (spring)	2.0	0.3

^a Source: Wallace *et al.* (1987)

Note: All differences between smokers and non-smokers are significant at $p < 0.001$.

Smokers were estimated to receive approximately 8 μg ethylbenzene per cigarette (Wallace *et al.*, 1987).

The consistency of the relationship between levels of ethylbenzene in exhaled breath and ambient air for non-smokers, reported by Wallace *et al.* (1996, 1997), was compared to the

relationship expected based on PBPK modeling (Krishnan, 2001; Appendix R). Based on the range of reported exhaled breath concentrations of ethylbenzene, approximate exposure concentrations of 1.54 to 10.3 $\mu\text{g}/\text{m}^3$ were derived, whereas the field measurements range from 2.1 to 8 $\mu\text{g}/\text{m}^3$. Thus the model predictions were consistent with the field measurements, within ~30 percent.

6.1.2 Blood

6.1.2.1 Non-Occupational Studies

Levels of several VOCs, including ethylbenzene, were measured in blood samples from a subset of adult participants (n = 982, aged 20 – 59)³ in the Third National Health and Nutrition Evaluation Survey (NHANES III) during the first cycle (1988 – 1991) and the first part of the second cycle (1992 – 1994) (Ashley *et al.*, 1992, 1994, 1995). The survey was designed to obtain nationally representative information about the general U.S. population rather than occupationally exposed individuals. The subsample examined for VOC levels included both men and women, and had the following demographic characteristics: 40% lived in the South (although all four regions of the country were represented), more than 50% lived in rural settings, 43% were low-income (annual income less than \$20,000), and approximately 60% were non-white.

Ethylbenzene was detectable in more than 75% of the blood samples collected (detection limit = 0.02 $\mu\text{g}/\text{L}$). Median, mean, and 95th percentile levels for the entire adult population were approximately 0.06, 0.11, and 0.25 $\mu\text{g}/\text{L}$ (Ashley *et al.*, 1994; Needham *et al.*, 1995; Churchill *et al.*, 2001). Levels for men and women aged 19 to 45, representing the reproductive years, are summarized in Table 6-2.

Table 6-2. Concentrations ($\mu\text{g}/\text{L}$) of Ethylbenzene in the Blood of U.S. Young Adults (Ages 19 to 45)^a

Sex	Number	Median	Mean	95 th Percentile	Range
Female	209	0.052	0.0814	0.175	0.014 - 1.789
Male	251	0.071	0.143	0.418	0.014 - 3.731

^a Source: NHANES III NCHS (2004)

Churchill *et al.* (2001) used odds ratios and 95% confidence intervals to examine the association between various exposures and the categorical outcome of having a VOC level above or below the 90th percentile of the distribution. As indicated in Table 6-3, ethylbenzene levels were increased in people who had reported recent use of paints, varnishes, or stains, who drank more than 20 ml of alcohol daily, and who smoked more than 20 pack-years (Churchill *et al.*, 2001). The association with alcohol consumption was also

³ Ethylbenzene concentrations in blood were only available for individuals between the ages of 20 and 59 years of age. Concentrations for ages 20 to 45 years were used as representative of the reproductive years of 19 to 45. Although men may be reproductively successful past the age of 45, their typical reproductive span would fall between 19 and 45. The range of blood concentration levels, 0.014 to 3.73 $\mu\text{g}/\text{L}$ (see Table 6-2), given for 19 to 45 year old men is also consistent for men between the ages of 20 and 59 years of age as reported in the NHANES database. The mean, median, and 95th percentile values of 0.134, 0.071, and 0.314 are similar.

observed for benzene, toluene, and styrene, compounds that are known to be present in tobacco smoke and elevated in smokers (*e.g.*, Wallace *et al.*, 1986). On this basis, Churchill *et al.* (2001) suggested that the typical co-location of smoking and drinking activities, *i.e.*, in bars and nightclubs, might explain the association of elevated levels of these aromatic compounds with alcohol consumption. Further, Churchill *et al.* (2001) reported that ethylbenzene levels, unlike those of xylenes, were not associated with recent exposure to gasoline. This finding may reflect the lower levels of ethylbenzene in gasoline.

Table 6-3. Multivariate Odds Ratios (and 95% Confidence Intervals) for Having a Blood Ethylbenzene Concentration Above or Below the 90th Percentile from Logistic Models for Ethylbenzene in Consumer Products and Geographic Location for 982 NHANES III Participants ^a

Exposure Factor	Odds Ratio	95% Confidence Intervals
Varnish, stain, or paint	3.0	1.2, 7.7
Toilet bowl deodorant	2.4	0.9, 6.0
>0-5 ml alcohol	1.1	0.5, 2.5
>5-20 ml alcohol	1.4	0.6, 3.3
>20 ml alcohol	3.8	1.3, 11.1
>0-5 pack-years smoked	1.3	0.5, 3.3
>5-20 pack-years smoked	1.8	0.8, 4.0
>20 pack-years smoked	3.1	1.3, 7.4
Male	2.0	0.9, 4.4
Live in Midwest	1.5	0.5, 4.5
Live in South	2.6	1.0, 6.5
Live in West	0.4	0.1, 1.4
Nail polish or polish remover used	0.1	0.0, 1.0
Fireplace used in past year	0.4	0.1, 1.2
^a Source: Churchill <i>et al.</i> (2001)		
Statistically significant		

Several studies have documented slight differences in blood levels of ethylbenzene in smokers vs. non-smokers (Table 6-4). It is not clear why the concentrations reported by Hajimiragha *et al.* (1989) are higher than those reported by Ashley *et al.* (1995) and Perbellini *et al.* (2002).

Table 6-4. Summary of Studies Comparing Blood Concentrations of Ethylbenzene in Smokers vs. Non-Smokers (µg/L)

Smoking Category	Mean	Median	Minimum	Maximum	Reference
Non-Smokers	0.651	0.431	0.175	2.284	Hajimiragha <i>et al.</i> (1989)
Smokers	0.837	0.533	0.378	2.697	
Non-Smokers	0.1	0.048	<0.02	2.7	Ashley <i>et al.</i> (1995)
Smokers	0.17	0.16	0.036	0.88	
Non-Smokers	0.222	0.145	nd	0.496	Perbellini <i>et al.</i> (2002)
Smokers	0.243	0.148	0.063	0.596	

Blood levels of ethylbenzene and other VOCs were not elevated relative to referents in a population living near an industrial complex in Kentucky, suggesting that proximity to industrial manufacturing and disposal facilities was not an important source of exposure (Hamar *et al.*, 1996).

The School Health Initiative: Environment, Learning, Disease (SHIELD) study examined children’s exposure over two years to complex mixtures of environmental agents, including ethylbenzene and other VOCs, environmental tobacco smoke (ETS), metals, pesticides, and allergens (Sexton *et al.*, 2005). Participants were selected from children in grades two through five from two economically disadvantaged and ethnically diverse neighborhoods in Minneapolis. Blood concentrations of ethylbenzene and other VOCs were measured up to four times over the two years (Table 6-5).

Table 6-5. Distribution of Ethylbenzene Concentrations in SHIELD Children’s Blood ($\mu\text{g/L}$)^a

Month/Year	Percentile of Distribution						
	10	25	50	75	90	95	99
Feb 2000	0.02	0.02	0.03	0.05	0.07	0.08	0.12
May 2000	0.01	0.02	0.03	0.04	0.05	0.07	0.17
Feb 2001	0.02	0.02	0.02	0.03	0.03	0.03	0.04
May 2001	0.03	0.04	0.05	0.06	0.08	0.09	0.10
Average	0.02	0.03	0.03	0.05	0.06	0.07	0.11

^a Source: Sexton *et al.* (2005)

No significant difference was observed between boys and girls, or among ethnic groups. Between- and within-child variability was similar. As shown in Table 6-5, ethylbenzene levels in the children’s blood (mean = 0.04 $\mu\text{g/L}$, median = 0.03 $\mu\text{g/L}$, 95th percentile = 0.07 $\mu\text{g/L}$) were two or more times lower than those of the adults who donated blood for NHANES III (mean = 0.11 $\mu\text{g/L}$, median = 0.06 $\mu\text{g/L}$, 95th percentile = 0.25 $\mu\text{g/L}$). This difference may be due to the declining levels of ethylbenzene in ambient air from the date (1988) the NHANES study began and this more current study, or to differences in pharmacokinetics between adults and children, or to less exposure (or usage) of consumer products (paints, etc.) or ETS. A somewhat unexpected result was the lack of correlation of ethylbenzene levels (and those of several other VOCs) in the children’s blood with personal air measurements, smoking in the home, or urinary cotinine (a marker for tobacco smoke exposure). This discrepancy may be due to suboptimal matching of the personal air and blood samples. Of the variables examined, only use of deodorizers in the six months preceding the study was associated with increased ethylbenzene levels.

The consistency of the relationship between levels of ethylbenzene in blood and ambient air in a limited number of biomonitoring studies was compared to the relationship expected based on PBPK modeling (Krishnan, 2001; Appendix R). The model predictions of blood concentration were found to be consistent with the field measurements (Mannino *et al.*,

1985) and NHANES data were consistent with ambient levels of ethylbenzene (Ashley *et al.*, 1994; Wallace *et al.*, 1986, 1987)

6.1.2.2 Occupational Studies

No information was located regarding concentrations of ethylbenzene in the blood of workers engaged in ethylbenzene production. Knecht *et al.* (2000) measured the concentrations of ethylbenzene in blood of 18 adult male (n = 12) and female (n = 6) volunteers aged 22 – 50 years during and after an eight-hour exposure to 25% or 100% of the German occupational standard (104,000 µg/m³ or 435,000 µg/m³) in a closed exposure chamber. After the higher exposure, mean ethylbenzene blood levels were 830 ± 210 µg/L, and the 95th percentile concentration was 1.12 mg/L. The mean concentration in blood following the lower exposure (determined from Figure 2 of the study) was approximately 200 µg/L. As the exposure levels in this study were much higher than any reported in styrene manufacturing facilities (see Section 6.3.2.1), it is very unlikely that workers in such facilities would have blood concentrations of this magnitude.

6.1.3 Estimating Concentrations of Ethylbenzene in Human Milk Using a Physiologically-Based Pharmacokinetic Model

There are few data available concerning transfer of ethylbenzene from maternal tissues to the fetus, or to young infants via breastfeeding. Pelizzari *et al.* (1982) detected ethylbenzene in 8 of 12 samples of human milk, but levels were not reported. Although monitoring data are limited, it is reasonable to believe that VOCs, including ethylbenzene, would be present in human milk (*e.g.*, Fisher *et al.*, 1997; Needham and Wang, 2002).

A PBPK model was used to estimate concentrations of ethylbenzene in the milk of nursing mothers (Sweeney and Gargas, 2006). Women in the general population and women who worked in ethylbenzene production facilities were considered. The model not only considered physiological parameters specific for nursing mothers but also incorporated the daily schedule of nursing and eating and time spent at home, in a vehicle, out of doors, and, for the production worker, time spent at work. Dietary intake of ethylbenzene was considered for the at-home mother but not the production worker because it was assumed that the intake from the diet would be small in comparison to workplace exposure. Further, the at-home mother modeled was assumed to smoke and live in an urban area, which is the most highly exposed group among the general population of women. As shown in Table 6-6, the central tendency and upper bound ethylbenzene concentrations for the at home mother were 0.11 µg/L and 0.25 µg/L, while the estimates for the occupationally exposed mother were 2.2 µg/L and 21.0 µg/L (Sweeney and Gargas, 2006).

Table 6-6. Estimated Central and Upper-Bound Concentrations of Ethylbenzene in Human Milk

	Concentration of Ethylbenzene in Mother's Milk (µg/L) ^a
General Public	
Central	0.11

Upper	0.25
Occupational Exposure	
Central	2.2
Upper	21.0
Source: Sweeney and Gargas, 2006	

6.1.4 Summary

Members of the general public with no occupational exposure to ethylbenzene, including children, have levels of ethylbenzene in blood in the parts-per-trillion range. Several studies in adults have shown that smoking increases ethylbenzene exposure (Wallace *et al.*, 1986, 1987), but its influence on mean blood levels in adults was not marked (Ashley *et al.*, 1995; Perbellini *et al.*, 2002), and was not apparent at all in the children studied by Sexton *et al.* (2005). Use of ethylbenzene-containing products increased levels in blood, but casual exposure to gasoline (Churchill *et al.*, 2001) and proximity to manufacturing and disposal sites (Hamar *et al.*, 1996) appeared to have no discernible effect. Recent use of paints, varnishes, or stains was associated with increased levels of ethylbenzene in blood, as was smoking and the consumption of 20 ml/day of alcohol (Churchill *et al.*, 2001).

A PBPK model was used to develop conservative estimates of ethylbenzene concentrations in human milk. The estimated concentrations in the milk of non-occupationally exposed women were 0.11 and 0.25 µg/L, based on the central tendency and upper bound blood limits, respectively. The estimates for the occupationally exposed women were 2.2 and 21.0 µg/L, respectively (Sweeney and Gargas, 2006).

6.2 Ethylbenzene Levels in Ambient Media

In this section, available data on ethylbenzene presence in ambient media are reviewed and evaluated for use in the exposure assessment.

6.2.1 Occurrence at Hazardous Waste Sites

Ethylbenzene has been identified in a variety of environmental media (air, soil gas, surface water, groundwater, leachate, soil, and sediment) collected at 720 of the 1,467 National Priority List (NPL) hazardous waste sites (ATSDR, 1999). The frequency of ethylbenzene detections in air, soil gas, surface water, groundwater, soil and sediment as of 1998 is shown in Figure 6-1 (from ATSDR, 1999). More recently, ethylbenzene ranked 39th on the Agency for Toxic Substances and Disease Registry's (ATSDR) 2003 list of substances most frequently found in completed exposure pathways at hazardous waste sites, present at 38 of 1,636 NPL sites and 62 of 4,791 total sites included in ATSDR's Completed Exposure Pathway (CEP) list (ATSDR 2003).

6.2.2 Ethylbenzene Levels in the Outdoor Environment

6.2.2.1 Ambient Air

As discussed in Section 5.2.2, the predominant environmental fate of ethylbenzene is volatilization to the atmosphere. Available data have demonstrated a relationship, assumed to be causal, between motor vehicle emissions and ambient ethylbenzene concentrations.

The initial TEAM studies (Wallace *et al.*, 1987) showed that ethylbenzene concentrations in urban California and New Jersey areas were higher than those in suburban Greensboro, North Carolina and rural Devils Lake, North Dakota areas (Table 6-7), suggesting a relationship with traffic density.

Table 6-7. Population-Weighted Mean Outdoor Ethylbenzene Concentrations Detected in TEAM Studies ^a

Location	Time Period	Mean Ethylbenzene Concentration ($\mu\text{g}/\text{m}^3$)
Bayonne-Elizabeth, NJ	9/81 – 11/81	4.0
	7/82 – 8/82	3.2
	2/83	3.8
Los Angeles, CA	2/84	9.7
	5/84	3.0
Contra Costa, CA	6/84	0.9
Greensboro, NC	5/82	0.3
Devils Lake, ND	10/82	0.03

^a Source: Wallace *et al.* (1987)

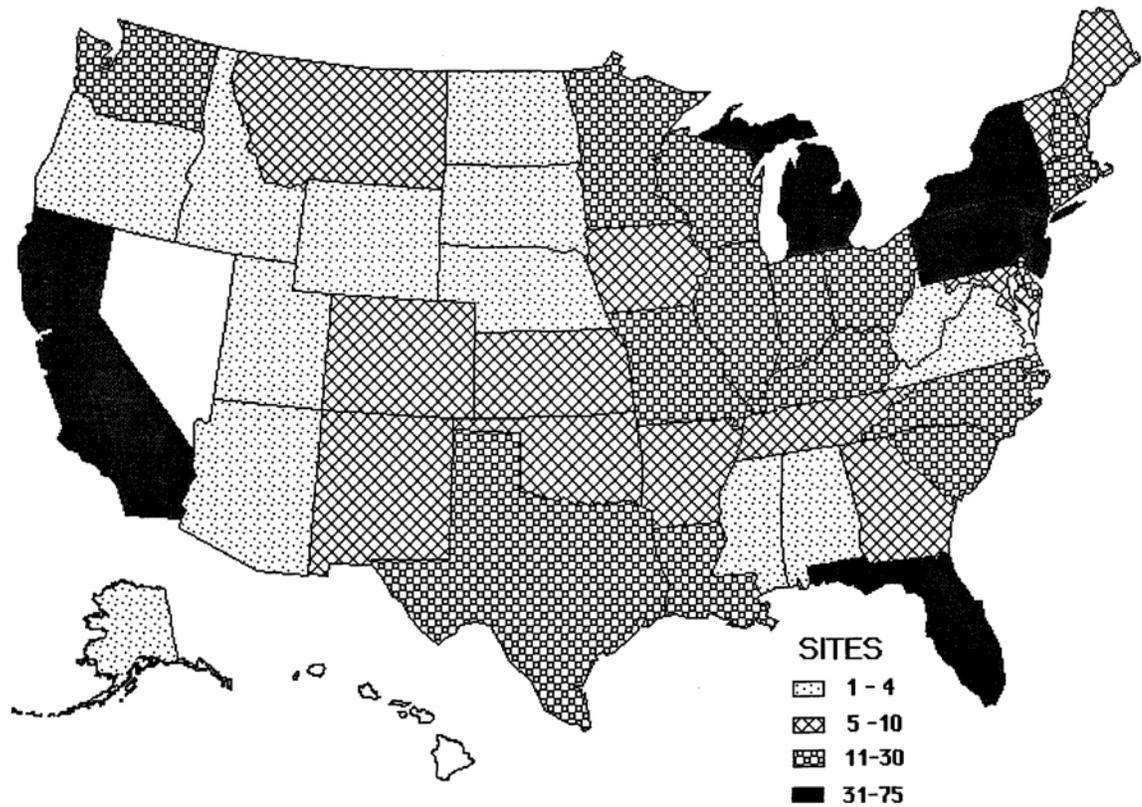


Figure 6-1. Frequency of NPL Sites with Ethylbenzene (from ATSDR, 1999)

In a review of factors influencing VOC concentrations in the U.S., Mohamed *et al.* (2002) also found a strong relationship between motor vehicle emissions and ambient levels of ethylbenzene and other gasoline-related aromatics. Ethylbenzene concentrations collected in rural areas ranged from 1.3 to 3.8 $\mu\text{g}/\text{m}^3$. A larger set of data has been obtained in cities with ranges of the mean and median concentrations being 0.3 to 71.5 $\mu\text{g}/\text{m}^3$ and 1.3 to 28.2 $\mu\text{g}/\text{m}^3$, respectively Mohamed *et al.* (2002). Older studies generally reported higher values (Mohamed *et al.*, 2002).

Similar results were obtained in a study in which mean ethylbenzene concentrations detected in samples collected from 13 semi-rural to urban locations in Maine, Massachusetts, New Jersey, Pennsylvania, Ohio, Illinois, Louisiana, and California ranged from 0.13 to 1.61 $\mu\text{g}/\text{m}^3$ (Pankow *et al.*, 2003). One- to 24-hour samples were collected over a period of seven to 29 months between April 1997 and September 2000. These data are summarized in Table 6-8. Median ethylbenzene concentrations were below 0.5 $\mu\text{g}/\text{m}^3$ at all locations except the three in urban California. Ethylbenzene levels were highly correlated with levels of other gasoline components in this data set (Pankow *et al.*, 2003).

Table 6-8. Ethylbenzene Concentrations Detected in Outdoor Air at 13 U.S. Locations^a

Location	# of Samples	Ethylbenzene Concentration ($\mu\text{g}/\text{m}^3$)		
		Mean	Median	Max
N. Windham, ME	20	0.48	0.39	1.56
Winchester, MA	7	0.52	0.22	2.17
Watertown, MA	4	0.48	0.16	1.43
Coles Farm, NJ	35	0.17	0.16	0.69
Rowan College, NJ	63	0.37	0.3	1.52
Turnersville, NJ	56	0.52	0.43	2.08
Traymore, PA	11	0.2	0.1	0.65
Kettering, OH	15	0.61	0.42	2.3
Western Springs, IL	15	0.13	0.42	2.3
Baton Rouge, LA	10	0.25	0.17	0.82
San Bernardino, CA	9	1.52	0.95	3.6
Anaheim, CA	9	1.61	1.65	3.3
Roseville, CA	17	0.65	0.53	2

^a Source: Pankow *et al.* (2003)

EPA's Urban Air Toxics Monitoring Program (UATMP) is designed to characterize the magnitude and composition of potentially toxic air pollution in or near urban locations. In monitoring conducted in 2003, ethylbenzene was detected 943 times at concentrations ranging from >0.04 to 12.4 $\mu\text{g}/\text{m}^3$ (EPA, 2004a). The mean, median, and standard deviation of this data set were 1.0, 0.65, and 1.2 $\mu\text{g}/\text{m}^3$, respectively (EPA, 2004a).

The EPA's Air Quality System (AQS) database (EPA, 2005a), accessible through the AirData page (<http://www.epa.gov/air/data/info.html>), provides access to yearly summaries of data collected from all fifty states plus the District of Columbia, Puerto Rico, and the U.S. Virgin Islands. The mean ethylbenzene concentrations recorded between 1994 and 2003

reveals a decreasing trend with time at urban and suburban locations, and a fairly consistent mean ethylbenzene concentration ($0.2 \mu\text{g}/\text{m}^3$) in rural areas (Figure 6-2).

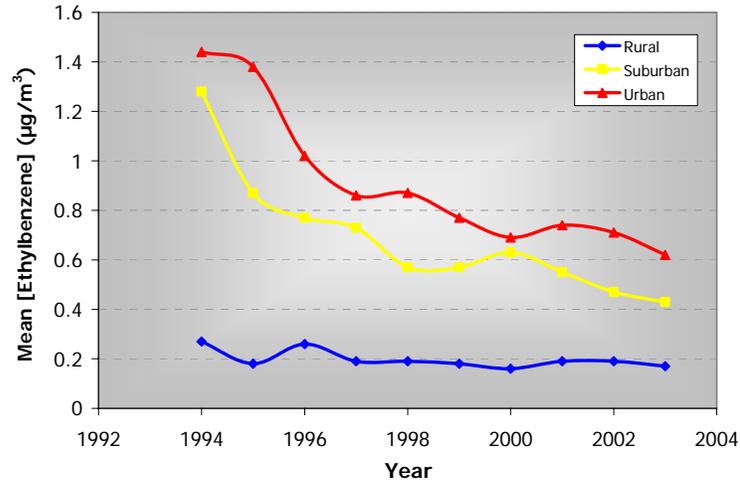


Figure 6-2. Mean Ethylbenzene Concentrations Measured at Urban, Suburban and Rural Locations Between 1994 and 2003 (from AQS database)

A parallel decreasing trend has also been observed in motor vehicle emissions of ethylbenzene and other fuel-related VOCs over the past decade, as shown in Figure 6-3 (Cook *et al.*, 2004).

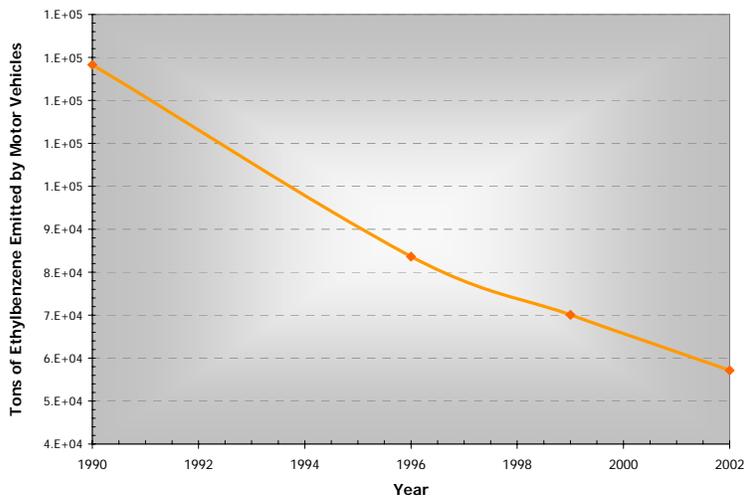


Figure 6-3. Reductions in Gaseous Ethylbenzene Emissions from Motor Vehicles (data from Cook *et al.*, 2004)

Ethylbenzene concentrations reported in EPA’s AQS database for the years 2000 through 2004 (excluding 2002, as data from this year appeared to include incorrect entries) are summarized by monitoring location (urban or suburban/rural) in Table 6-9, with the raw data provided in Appendix E. Consistent with the other data sets, mean ethylbenzene concentrations were higher in urban areas compared to rural and suburban locations, as expected due to its association with automotive fuels and exhaust.

Table 6-9. Summary of Rural/Suburban and Urban Ethylbenzene Concentrations ($\mu\text{g}/\text{m}^3$) Reported in EPA’s Air Quality System Database, 2000 – 2004^{a,b}

Category	N	Summary of Arithmetic Means			Summary of 95 th Percentiles		
		Mean \pm SD	Minimum	Maximum	Mean \pm SD	Minimum	Maximum
Unknown	13	0.17 \pm 0.16	0.05	0.56	0.44 \pm 0.44	0.11	1.52
Rural/Suburban	589	0.59 \pm 0.77	0.01	9.38	1.54 \pm 2.87	0.003	43.35
Urban	443	1.09 \pm 6.21	0.05	130.08	2.43 \pm 7.39	0.09	130.08
Overall	1,045	0.80 \pm 4.09	0.01	130.08	1.90 \pm 5.29	0.003	130.08

^a Source: (<http://www.epa.gov/air/data/info.html>) (Appendix E)
^b Data from 2002 were excluded due to apparent data quality issues.

6.2.2.2 Potable Water

Data from a variety of sources were examined in order to characterize the presence of ethylbenzene in the potable water supply. Although groundwater and surface water resources can be affected by localized spills and other releases, available data consistently indicate low detection frequency and low concentrations of ethylbenzene in both groundwater and surface water resources used for drinking.

Groundwater

ATSDR’s Hazardous Substance Release/Health Effects Database (HazDat) database (Appendix F) reported detected concentrations of ethylbenzene in groundwater ranging from 0.2 to 23,000 $\mu\text{g}/\text{L}$ in private and municipal wells, reflecting impacted areas near sources of contamination such as landfills, waste sites, or gas stations (ATSDR, 2005). However, as this database represents impacted resources rather than widespread long-term conditions, it is not appropriate for characterizing chronic exposure to the general public.

Available groundwater data for ethylbenzene were obtained from the U.S. Geological Survey’s (USGS’s) National Water Quality Assessment Program (NAWQA) website <http://sd.water.usgs.gov/nawqa/vocns/> (Appendix G) (USGS, 2005). These data, summarized in Table 6-10, include 6,324 records collected between September 1996 and September 2003 from sites in almost all 50 States, and categorized with respect to its primary use. As indicated in Table 6-10, ethylbenzene was detected in only around 2% of the samples.

Table 6-10. Summary of Groundwater Data for Ethylbenzene from the USGS National Water Quality Assessment Database ^a

Category	Number Samples	Minimum (µg/L)	Maximum (µg/L)	Mean (µg/L)	95% UCL ^b (µg/L)
Non-detects	6,202	0.03	100	-	-
Estimated	96	0.002	0.254	-	-
Detected	26	0.1	2,100	-	-
Non-potable	2,994	0.002	2,100	1.03	4.21
Potable ^c	3,330	0.003	5.4	0.042	0.05
All samples	6,324	0.002	2,100	0.51	2.02

^a USGS (2005).
^b Upper confidence limits are based upon a non-parametric Chevshev distribution
^c Classified as Commercial, Domestic, Industrial, Institutional, or Public Supply in the database

Although maximum ethylbenzene concentrations were as high as 2,100 µg/L in non-potable samples, concentrations were much lower in waters used for drinking. For wells identified as potable, the mean and 95% UCL were 0.042 µg/L (standard deviation = 0.1 µg/L) and 0.05 µg/L, respectively (calculated assuming non-detected concentrations to be equivalent to one-half the detection limit).

Data available from the National Contaminant Occurrence Database (NCOD) (<http://www.epa.gov/safewater/data/ncod.html>) were also reviewed (EPA, 2005b). The NCOD was developed to satisfy the statutory requirements set by Congress in the 1996 Safe Drinking Water Act (SDWA) amendments to maintain a national drinking water contaminant occurrence database using occurrence data for both regulated and unregulated contaminants in public water systems. Groundwater data within the database is segregated into Round 1 and 2 data and 6-Year Review Data. Round 1 and 2 data were collected between 1988 and 1997 in 35 to 40 states, while the 6-Year Review data were collected between 1993 and 1997 in 16 states. Table 6-11 summarizes the available groundwater data in the NCOD. Ethylbenzene was detected in less than 1% of the samples in both data sets. Of the groundwater-based public water supplies, 1.3% had ethylbenzene detections (EPA, 2001).

Table 6-11. Summary of Community Water Supply (Groundwater) Data for Ethylbenzene from the EPA National Contaminant Occurrence Database ^a

Data Group	No. of Analyses	Detection Frequency	Results (µg/L)		
			Minimum	Maximum	Mean
Round 1 & 2	44,812	0.6%	0.02	44	2.4
6-Year Review ^b	66,234	1.3%	10	390	3

^a EPA (2005b).
^b Reported minimum reporting limits ranged from 10 to 20 µg/L

Moran *et al.* (2002) evaluated samples taken from 1,926 rural, self-supplied domestic wells during 1986 – 1999. Ethylbenzene was detected only twice, for a detection frequency of 0.1%. The maximum detected concentration was 5.4 µg/L with the other detected concentration being 0.2 µg/L.

Similar results have been published in the peer-reviewed literature. In a NAWQA-related study of untreated groundwater from 2,948 potable and non-potable wells between 1985 and 1995, Squillace *et al.* (1999) detected ethylbenzene in 1.7% of urban wells (range = 0.2 – 51 µg/L, median = 1.9 µg/L) and 0.2% of rural wells (range = 0.2 – 270 µg/L, median = 2.8 µg/L). Ethylbenzene was the least frequently detected of the 14 most frequently detected VOCs in a study of groundwater from 581 shallow monitoring wells in new residential/commercial areas conducted between 1996 and 2002 as part of the NAWQA (Squillace *et al.*, 2004). When data were censored at 0.2 µg/L, the detection frequency for ethylbenzene was 0.4%, less than that in urban land-use areas, and greater than that in rural areas. Detected concentrations in these shallow (non-potable) wells ranged from 0.002 to 3.6 µg/L (Squillace *et al.*, 2004).

Surface Water

ATSDR’s HazDat database (Appendix F) contained detected concentrations of ethylbenzene in surface water (described as “lakes, streams, ponds, etc.”) ranging from 0.001 to 140,000 µg/L, reflecting impacted areas near sources of contamination such as landfills, waste sites, or gas stations (ATSDR, 2005). However, as this database represents impacted resources rather than widespread long-term conditions, it is not appropriate for characterizing chronic exposure to the general public.

Surface water data for ethylbenzene were obtained from NAWQA (<http://sd.water.usgs.gov/nawqa/vocns/>) (Appendix G) (USGS, 2005). These data consisted of 1,802 records collected between September 1993 and September 2003 from sites in the majority of the 50 states. Whether the sampled water bodies were used as potable water supplies was not specified. A summary of these data is presented in Table 6-12. Overall, ethylbenzene was not detected in the majority (1,517) of the samples taken – 1,517/1,802 = 84%. Estimated detected concentrations were provided for 267 of the samples, with 18 results containing no remarks and assumed to represent detected concentrations. Detected concentrations ranged from 0.42 µg/L to 3 µg/L. The mean concentration of ethylbenzene over all samples, with the non-detected concentrations assumed to be equivalent to one-half the detection limit, was 0.045 µg/L, with a 95% UCL of 0.06 µg/L.

Table 6-12. Summary of Surface Water Data for Ethylbenzene from the USGS National Water Quality Assessment Database ^a

Category	Number Samples	Minimum (µg/L)	Maximum (µg/L)	Mean (µg/L)	95% UCL ^b (µg/L)
Non-detects	1,517	0.03	0.8	-	-
Estimated	267	0.002	0.132	-	-
Detected	18	0.419	3	-	-
All samples	1,802	0.002	3	0.045	0.064

^a USGS (2005)

^b Upper confidence limits are based upon a non-parametric Chevyshev distribution

The USGS conducted a survey of VOCs in 954 randomly selected community drinking water supplies, both groundwater and surface water, from May 1999 to October 2000 (Grady, 2003). Gasoline-related VOCs were detected slightly more frequently in surface water than in groundwater, although the difference was not statistically significant, and detection frequency was significantly related to urban land use and gasoline storage tank density (Grady, 2003). Summary statistics for surface water supplies are presented in Table 6-13. These data show that ethylbenzene was detected in less than 1% of samples, and never in river water. The maximum detected concentration was 1 µg/L.

Table 6-13. Frequency of Detection and Concentrations of Ethylbenzene in Potable Surface Water Supplies ^a

Statistic	Source Water		
	Surface Water	River	Reservoir
Number of samples	373	170	203
Detection frequency (%)	0.54	0	0.98
Minimum (µg/L)	0.26	<0.2	0.26
Maximum (µg/L)	1	<0.2	1

^a Source: Grady (2003)

A review of surface water data available from the NCOD (Appendix H) was also conducted. A summary of the surface water data, presented in Table 6-14, indicates that ethylbenzene was infrequently detected (less than 1% of the Round 1 & 2 data and in 1.2% of the 6-Year Review data).

Table 6-14. Summary of Community Water Supply (Surface Water) Data for Ethylbenzene the EPA National Contaminant Occurrence Database ^a

Data Group	Number Analyses	Detection Frequency	Results (µg/liter)		
			Minimum	Maximum	Mean
Round 1 & 2	7,981	0.9%	0.01	100	4.7
6-Year Review ^b	12,160	1.2%	10	20	1

^a Accessed November 2004
^b Reported minimal reporting limits ranged from 10 to 20 µg/liter

6.2.2.3 Soil

As discussed in Section 5.2.2, only small amounts of ethylbenzene are expected to be found in soil on account of its high volatility, low soil sorption (low K_{oc}), and relatively rapid breakdown by soil bacteria. ATSDR's HazDat database (Appendix F) reported detected concentrations of ethylbenzene in soil (all depths) ranging from 0.0009 to 180,000 mg/kg, reflecting impacted areas near sources of contamination such as landfills, waste sites, or gas stations (ATSDR, 2005). However, as this database represents impacted resources rather

than widespread long-term conditions, it is not appropriate for characterizing chronic exposure to the general public.

A search of the EPA's Storage and Retrieval database (STORET) (<http://www.epa.gov/storet/dbtop.html>) revealed 389 soil samples analyzed for ethylbenzene, of which 66 had detected levels ranging from 0.0014 to 31 mg/kg (EPA, 2005d). However, all of the samples were taken at the same site in Utah, and as such cannot be considered representative of the entire United States.

6.2.2.4 Sediment

As discussed in Section 5.2.2, ethylbenzene is not expected to sorb appreciably to sediment. ATSDR's HazDat database (Appendix F) reported detected concentrations of ethylbenzene in sediment (described as "lakes, streams, ponds, etc.") ranging from 0.004 to 270 mg/kg (ATSDR, 2005), reflecting impacted areas near sources of contamination such as landfills, waste sites, or gas stations. However, as this database represents impacted resources rather than widespread long-term conditions, it is not appropriate for characterizing chronic exposure to the general public.

The STORET database reported detectable levels of ethylbenzene in 12 of 335 sediment samples, with levels ranging from 0.0012 mg/kg to 55 mg/kg (EPA, 2005d). These data were collected at a limited number of sites in various states.

6.2.3 Summary

6.2.3.1 Ambient Air

Given continuing input of ethylbenzene from multiple sources (see Section 5.3), inhalation of the compound is expected to be a consistent human exposure route. Although concentrations of ethylbenzene and other VOCs in ambient air have been trending downward for a number of years (Figure 6-2) (EPA, 2003), in parallel with decreases in motor vehicle emissions (Figure 6-3) (Cook *et al.*, 2004), concentrations remain higher in urban than non-urban areas.

The most comprehensive and up-to-date source of data for air-related risk assessment is EPA's AQS. The averaged data for the past five years presented in Table 6-9 provide a sound basis for estimating central tendency (mean) and upper-bound (95th percentile) exposure concentrations for urban and non-urban dwellers. For urban areas, the selected central tendency and upper-bound values are 1.09 $\mu\text{g}/\text{m}^3$ and 2.43 $\mu\text{g}/\text{m}^3$, respectively, while for rural/suburban areas, the central tendency is 0.59 $\mu\text{g}/\text{m}^3$ and the upper-bound is 1.54 $\mu\text{g}/\text{m}^3$.

6.2.3.2 Potable Water

As discussed in Section 6.2.2.2, ethylbenzene was very rarely detected in multiple national surveys of potable groundwater and surface water resources, and never at concentrations approaching the Maximum Contaminant Level (MCL)⁴. These data indicate that the

⁴ An MCL is defined as the highest level of a contaminant that EPA allows in drinking water. MCLs ensure that drinking water does not pose either a short-term or long-term health risk. EPA sets MCLs at levels that are economically and technologically feasible. The MCL for ethylbenzene is 0.7 mg/liter.

populations of interest in this exposure assessment are not regularly exposed to measurable levels of ethylbenzene in their drinking water. Although localized contamination can occur in connection with specific sources, there is no basis for estimating concentrations representative of chronic exposure conditions for such populations. Therefore, exposures by way of potable water are considered negligible and are not considered quantitatively in this exposure assessment.

6.2.3.4 Soil/Sediment

Fugacity modeling predicts that very little ethylbenzene will partition to soil or sediment (Table 5-6), and its physicochemical properties ensure its mobility in these media (leaching, solubilization, volatilization). It is therefore not surprising that few data pertaining to ethylbenzene concentrations in soil and sediment could be located. The only levels reported in soil and sediment were from a limited number of hazardous waste sites, and as such are not suitable for the estimation of chronic exposure to the general public. Based on this information, exposure of the general population to soil-associated ethylbenzene is considered negligible, and is not considered quantitatively in this exposure assessment.

6.3 Ethylbenzene Levels in Indoor Air

The indoor environment has become a major venue for exposure assessment not only because most individuals, including children, spend the majority of their time indoors (Klepeis *et al.*, 2001), but also because concentrations of most VOCs are typically higher in buildings than in ambient air. The EPA's TEAM studies and other large-scale investigations of personal exposure to environmental chemicals among the general population in the U.S., Western Europe, and Australia have provided much information about the sources and magnitude of exposure to ethylbenzene and other VOCs in the indoor environment.

Indoor air quality is a complex function of a building's location, characteristics, composition, content, and uses. The most prominent determinants are the volume of air in the building, the emission/removal rate of the VOCs in the indoor microenvironment, the building ventilation rate, and outdoor concentrations (*e.g.*, Kim *et al.*, 2001). Available data indicate that factors that may contribute to ethylbenzene levels in buildings include: (1) traffic density; (2) the presence of attached garages or structures where gasoline or kerosene may be stored; (3) ETS; (4) the presence or use of certain household products (cleaners, marking pens, paints, glues, etc.); and, (5) styrenic building and furnishing materials.

In this section, available information is reviewed to identify appropriate concentrations of ethylbenzene in various indoor microenvironments for use in this Tier 1 exposure assessment. As the data presented do not permit its segregation into that attributable to the ethylbenzene/styrene chain of commerce, refinery chain of commerce, or other sources, the results are presented as ethylbenzene concentrations in indoor air. A procedure for assigning a percentage of these concentrations to the major sources is described in Section 6.7.5.

6.3.1 Ethylbenzene Levels in Homes

A national VOC Database compiled by Shah and Singh (1988) in the late 1980s contains more than 52,000 records representing indoor air measurements from 30 different cities in 16 states (although almost 90% of the data are from California and New Jersey). The data include samples collected prior to 1986 from both residential and workplace environments, as well as personal exposures. At that time, ethylbenzene concentrations in indoor air averaged $12.5 \mu\text{g}/\text{m}^3$, with a median concentration of $4.8 \mu\text{g}/\text{m}^3$. A 1994 review of available international data reported similar values, with a weighted average geometric mean ethylbenzene concentration in indoor air of $5 \mu\text{g}/\text{m}^3$ and 90th percentile of $22 \mu\text{g}/\text{m}^3$ based on 1,867 measurements from 1,225 dwellings (Brown *et al.*, 1994).

A more recent survey reviewed indoor air concentrations detected in North America since 1990 (Hodgson and Levin, 2003). The median ethylbenzene concentration reported, based on data from four studies ($n = 160$), was $2.3 \mu\text{g}/\text{m}^3$ – about 50% lower than the median concentration reported by Shah and Singh (1988) and the geometric mean reported by Brown *et al.* (1994). Figure 6-4 presents a comparison of data presented in Pellizzari *et al.* (1986) which summarized the 1980-1984 EPA TEAM studies data to the data presented in Hodgson and Levin (2003). While it can be inferred from this comparison that, as in ambient air (see Figure 6-2), ethylbenzene concentrations in indoor air have decreased in recent years (Figure 6-4) one must consider the limited set of data ($n=160$) in (Hodgson and Levin, 2003) compared to the 52,000 records in Shah and Singh (1988).

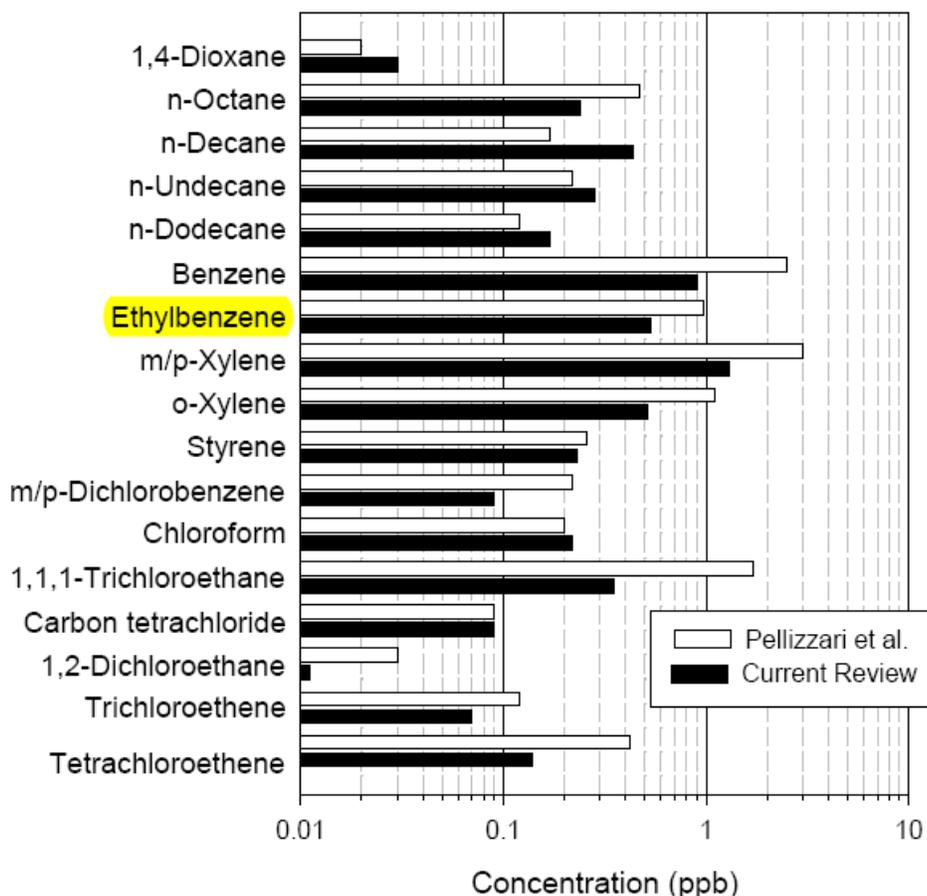


Figure 6-4. Comparison of Central Tendency VOC Concentrations in Existing Residences in Hodgson and Levin (2003) with Geometric Mean Concentrations from the 1980-1984 EPA TEAM Studies (from Hodgson and Levin, 2003)

6.3.1.1 Automotive Sources

As noted previously, automotive and other petroleum-related sources have been recognized as major sources of ethylbenzene and other VOCs in both ambient and indoor air (see Section 6.2.3.1). Data from the TEAM studies have been analyzed using positive matrix factorization in order to elucidate sources (Anderson *et al.*, 2001). The main source of personal exposure to ethylbenzene and other aromatics in New Jersey and California was determined to be vapors and automobile exhaust (as well as ETS).

In a comparative study of VOC concentrations and sources in rural vs. urban German homes, Ilgen *et al.* (2001b) observed that indoor ethylbenzene levels were dominated by traffic-related sources in urban areas, but the influence of indoor sources was noted in rural areas. Thus, the indoor to outdoor (I/O) ratio was 6.2 in the rural area, but only 1.2 in urban rooms facing the street.

Ilgen *et al.* (2001a) reported that having an attached garage contributed to indoor levels of ethylbenzene. A study of a single home included within their report showed that

ethylbenzene concentrations in the living room of the home increased by a factor of 2 when a car was stored within the attached garage compared to when one was not present.

Ethylbenzene concentrations detected in ten Southeast Chicago homes without attached garages and occupied by non-smokers between June, 1994 and April, 1995 ranged from 0.91 to 174 $\mu\text{g}/\text{m}^3$ with a mean of 9.7 $\mu\text{g}/\text{m}^3$ and median of 3.21 $\mu\text{g}/\text{m}^3$ ($n = 48$; 90th percentile = 13.0 $\mu\text{g}/\text{m}^3$) (Van Winkle and Scheff, 2001). The median ethylbenzene concentration outside these homes was 0.63 $\mu\text{g}/\text{m}^3$ (I/O ratio = 5). The authors noted that significantly higher concentrations of ethylbenzene were found when air conditioners were in use, and that higher ethylbenzene concentrations were associated with the storage of chemicals (miscellaneous cleaners) in the kitchen.

The TEACH (Toxic Exposure Assessment, a Columbia/Harvard) study was designed to assess personal exposures to urban air toxics among non-smoking inner-city high school students living in New York City and Los Angeles. Results from the New York City phase of the TEACH study were reported by Kinney *et al.* (2002). Indoor, outdoor, and personal air samples (48-hour averaging time) were collected during the summer and winter of 1999 for a group of 46 high school students attending a public high school in the West Central Harlem section of New York City. Mean ethylbenzene concentrations in indoor, outdoor, and personal air samples were 1.99, 1.88, and 3.37 $\mu\text{g}/\text{m}^3$, respectively, in summer, and 3.57, 1.27, and 2.24 $\mu\text{g}/\text{m}^3$, respectively, in winter. The average of winter and summer mean values was 2.37 $\mu\text{g}/\text{m}^3$. As expected, the I/O ratio was greater in winter than in summer ($3.57/1.27 = 2.8$ vs. $1.99/1.88 = 1.1$), although not greatly. The authors suggested that the similarity of the indoor, outdoor, and personal exposure values may be indicative of permeation of buildings by outdoor sources (primarily motor vehicle emissions).

Sax *et al.* (2004) compared indoor and outdoor levels of VOCs from the same study, reporting slightly different results for New York City. These data are presented in Table 6-15. Median concentrations in indoor and outdoor air were similar, leading the authors to conclude that outdoor automotive sources were the primary source of ethylbenzene in indoor and outdoor air in the study.

Table 6-15. Comparison of Indoor and Outdoor Ethylbenzene Concentrations ($\mu\text{g}/\text{m}^3$) Detected in New York and Los Angeles ^a

Season	Location	Samples	Min.	Median	Max.	Mean	SD	Mean I/O Ratio
<i>New York (1999)</i>								
Winter	Indoor	36	0.5	1.6	34	3.2	6.1	2.9
	Outdoor	31	0.3	1	2	1.1	0.5	
Summer	Indoor	30	0.4	1.6	5.1	1.8	1	1.1
	Outdoor	26	0.5	1.3	8.9	1.7	1.6	
Average Indoor			0.45	1.6	19.55	2.5		
<i>Los Angeles</i>								
Winter	Indoor	40	1.4	2.8	7.5	3	1.5	1.0
	Outdoor	35	1	2.7	7.3	2.9	1.4	
Fall	Indoor	32	0.8	1.8	1.5	2.5	2.6	1.2
	Outdoor	32	0.9	2.1	3.4	2.1	0.7	
Average Indoor			1.1	2.3	4.5	2.75		
^a Source: Sax <i>et al.</i> (2004)								

Indoor air ethylbenzene concentrations averaged $3.22 \mu\text{g}/\text{m}^3$ (three-day, time-weighted averages) in three South Baltimore communities sampled in January 2000 – June 2001 (Payne-Sturges *et al.*, 2004). The average indoor air ethylbenzene level was similar ($3.9 \mu\text{g}/\text{m}^3$) in two-day, time-weighted average samples collected in 71 homes of non-smokers in the Minneapolis/St. Paul metropolitan area in spring, summer and fall, 1999 (Sexton *et al.*, 2004). Mean ethylbenzene concentrations in matched outdoor samples were lower than corresponding mean indoor air samples in both studies ($1.26 \mu\text{g}/\text{m}^3$ in South Baltimore and $0.7 \mu\text{g}/\text{m}^3$ in Minneapolis/St. Paul). These concentrations resulted in an I/O ratio of 2.6 ($3.22/1.26$) for the South Baltimore samples and 5.6 ($3.9/0.7$) for the Minneapolis/St. Paul samples. The mean ethylbenzene concentrations in personal air samples were somewhat higher at $4.42 \mu\text{g}/\text{m}^3$ in the South Baltimore study and $5.6 \mu\text{g}/\text{m}^3$ in the Minnesota study.

6.3.1.2 Household Products

Searches of the Source Ranking Database (EPA, 2004c) and the SACK Database (Sack, 2005) (Appendix I), both of which date from the 1980s, indicated that ethylbenzene was present in the following product categories:

- Auto products (cleaners and degreasers);
- Household products (spot removers, polish, and rugs and upholstery cleaners);
- Aerosol paints, paint thinners, primers, stains, and waterproofing compounds; and
- Insecticides and pesticides.

The widespread use of many of these products likely results in indoor exposure to a multitude of hydrocarbon substances including ethylbenzene. For example, Wallace *et al.* (1989) reported that painting and using a carburetor cleaner increased ethylbenzene exposure by 100-fold above background. Painting, varnishing, and other redecorating activities increased concentrations of ethylbenzene in German homes (Ilgen *et al.*, 2001a). Emissions

of both ethylbenzene and *o*-xylene in a study of ten urban homes were associated with storage of cleaners in kitchens (Van Winkle and Scheff, 2001). However, examination of the current National Library of Medicine Household Products Database indicates that ethylbenzene is no longer present in household cleaning products.

It is important to note that the ethylbenzene in these products is considered not to derive from the ethylbenzene/styrene chain of commerce; rather, it is generally petroleum-derived or a component of mixed xylenes. This is evident from a review of the specific products identified in Appendix I. In addition to the levels of ethylbenzene, the concentrations of total xylenes or *m*-xylene and *o,p*-xylene congeners is also presented in Appendices I1 to I3 to illustrate that the majority of the typical household products that contain ethylbenzene also contained mixed xylenes.

As shown in Table 6-16, which is representative of the data specified in Appendix I, the majority product categories listed as containing ethylbenzene also contained one of the xylenes (either *m*-xylene, *o,p*-xylene, or both) indicating that the ethylbenzene present was a component of mixed xylenes. Similarly, results of the Household Products Database search, listed in Appendix I, indicate that in virtually all cases when ethylbenzene was present xylenes also were present.

Table 6-16. Average Analyte Concentrations and Percentage Positive Hits from the Sack Database ^a

Category	% Containing			Average Concentration (%w/w)		
	Ethylbenzene	<i>m</i> -Xylene	<i>o,p</i> -Xylene	Ethylbenzene	<i>m</i> -Xylene	<i>o,p</i> -Xylene
Automotive products	7.5	26.7	10.0	7.2	10.6	31.0
Household cleaners/polishes	1.2	33.3	0	0.1	1.4	0
Paint-related products	47.8	60.3	58.2	2.4	4.2	2.8
Fabric and leather treatments	11.8	NA	33.3	1.0	NA	0.1
Cleaners for electronic equipment	4.5	NA	NA	0.1	NA	NA
Miscellaneous products	5	NA	NA	NA	NA	NA
NA – Not Available						
^a – Source: Sack (2005) (Appendix I).						

Since the primary focus of this evaluation is to address potential exposure to ethylbenzene within the ethylbenzene/styrene chain of commerce, no formal evaluation of the contribution to ethylbenzene in indoor microenvironments from household products was conducted. However, two scenarios involving acute exposure of children and prospective parents to mixed xylenes in consumer products (metal parts degreasing and spray painting) were quantitatively evaluated in the xylenes VCCEP submission (ACC, 2005). Modeled central tendency and upper-bound time-weighted average xylenes concentrations in indoor air for three time periods (one hour, eight hours, and 24 hours) ranged from 0.14 to 46 ppm. As indicated in Section 1.2, mixed xylenes may contain 6 to 15% ethylbenzene. Therefore, conservatively assuming that the xylenes comprise 85% and ethylbenzene 15% of mixed xylenes in the modeled consumer products, the range of total mixed xylene concentrations in these scenarios can be estimated as 0.2 to 54 ppm.

For long-term scenarios, it is assumed that any contribution from the use of household products would contribute to the indoor air concentrations presented in Section 6.3.1 and be taken into account in the total ethylbenzene concentration.

6.3.1.3 Styrenic Construction and Consumer Products

Recognition of the fact that building materials and furnishings can impact indoor air quality through off-gassing of VOCs has prompted investigation of these materials in recent years. However, there are few data specifically evaluating their potential contribution to indoor levels of ethylbenzene. The Source Ranking Database lists the building materials in Table 6-17 as sources of ethylbenzene (EPA, 2004c). While using these values to estimate indoor air is problematic, an emission rate is provided in the Source Ranking Database for these building materials. The experimental methods used in the determination of the emission rates, such as the duration of the study or the size of the area in which emissions were measured, are not reported. Assumptions regarding the area of home affected by the emissions, dilution of emissions (e.g., air exchange rates), and decreases in emissions over time would make estimates of indoor air concentrations highly uncertain. Any emissions from these products into the indoor air are expected to have been captured in the indoor air measurements presented in Section 6.3.1.

Table 6-17. Construction Materials Potentially Containing Ethylbenzene ^a

Product	Emission Rate ^b
Polypropylene and Networx [®] modular carpet	10
Wood office furniture	8
Other rubber floor and wall coverings including cove base, wainscoting, etc.	3
Polystyrene rigid foam insulation	13
Monokote [®] fireproofing	11
Sheet vinyl flooring	65
^a Source: Source Ranking Database (EPA, 2004c) (Appendix I)	
^b In units of $\mu\text{g}/\text{m}^2\text{-hr}$ for materials measured by area (e.g. carpet) or $\mu\text{g}/\text{unit-hr}$ for material measured as individual items (e.g., home furnishings).	

Results of studies measuring indoor air concentrations of ethylbenzene associated with building materials have been generally equivocal. Brown *et al.* (1994) noted that concentrations of VOCs in new homes, offices, schools, and hospitals were typically higher than those in established buildings, presumably due to off-gassing from building materials and contents. However, ethylbenzene was not among the predominant VOCs in new buildings (Brown *et al.*, 1994). Rather, ethylbenzene was predominantly associated with established dwellings (Brown *et al.*, 1994). Similarly, in a recent review of indoor air data since 1990, maximum ethylbenzene levels in existing homes ($47.8 \mu\text{g}/\text{m}^3$) were more than five times higher than those in new homes ($9.1 \mu\text{g}/\text{m}^3$) with the central tendency in existing homes ($2.3 \mu\text{g}/\text{m}^3$) approximately 60% more than that in new homes ($1.4 \mu\text{g}/\text{m}^3$) (Hodgson and Levin, 2003).

A principal component analysis of VOC sources in the EXPOLIS-Helsinki study showed that unlike styrene, ethylbenzene levels in residential indoor air were not associated with the

factor related to carpets, rubber, and adhesives (Edwards *et al.*, 2001). A comparative study of indoor air concentrations in urban and rural homes in Germany showed no correlation between building materials and furniture and any benzene, toluene, ethylbenzene, and xylenes (BTEX) compounds (Ilgen *et al.*, 2001a).

Ethylbenzene concentrations were measured in six experimental “enhanced indoor air quality homes” located in a rural Colorado community in 1992 and 1993 (Lindstrom *et al.*, 1995). These homes were built using low-emission materials, and none had attached garages. Data were collected over two consecutive 12-hour sampling intervals, pre- and post-occupancy, and compared to ethylbenzene levels measured in three conventional style homes with attached garages built at the same time. In the pre-occupancy phase, ethylbenzene was detected in two of six experimental homes at geometric mean levels of 3.41 and 3.33 $\mu\text{g}/\text{m}^3$, compared with a range of 1.49 to 9.15 $\mu\text{g}/\text{m}^3$ in the conventional homes. In follow-up measurements made five months after occupancy (by non-smokers in all cases), ethylbenzene was detected in all six experimental homes; however, geometric mean ethylbenzene concentrations were lower than previously, ranging from 0.33 to 1.4 $\mu\text{g}/\text{m}^3$. In contrast, concentrations of ethylbenzene and other fuel-related compounds increased following occupation in the conventional homes (geometric means of 2.41 to 7.04 $\mu\text{g}/\text{m}^3$), probably reflecting the influence of automotive sources in these homes’ attached garages. Taken together, these data suggest that in the absence of strong input from motor vehicle-related sources, low levels of ethylbenzene emissions from building materials may be discernible, at least for a time. However, the magnitude and duration of any such contribution is unknown, and apparently negligible in comparison with the contributions from petroleum-related sources that are usually present in developed areas.

Several studies have examined emissions of ethylbenzene and other VOCs from specific items or materials. In a recent survey of indoor sources of VOCs (Hodgson, 1999), ethylbenzene was only reported to be associated with one item, an older, subsequently discontinued carpet sample with SBR backing. Emissions from this carpet decreased rapidly with time. Similarly, Hodgson (2003) reported no ethylbenzene emissions from polystyrene-containing structural insulated panels, although there were transient emissions from several panel adhesives (not considered part of the chain of commerce for this exposure assessment).

Zellweger *et al.* (1997) characterized the emission behavior of a wide variety of building materials as well as furniture, fittings, and household products. They reported relatively low rates of ethylbenzene emission from a polyurethane foam, asphalt slabs, water-based parquet sealing, sealing for bitumen surfaces, a synthetic resin-based lacquer, a sealed table top, and a computer monitor. Of these items, only the computer monitor, the casing of which could be made of polystyrene, appears to be part of the chain of commerce.

Malmgren-Hansen *et al.* (2003) conducted a literature survey of emissions associated with electronic equipment used in Danish homes, including computers, monitors, game consoles, and audio and video systems. Ethylbenzene emissions were reported from a computer monitor manufactured in 1990 and a mobile phone; the maximum emission rate was 5.6 $\mu\text{g}/\text{hour}$. Malmgren-Hansen *et al.* (2003) also purchased four items in Denmark (a computer monitor, a television set, a game console, and several voltage converters for halogen lamps), measured VOC emissions in a test chamber after seven hours and nine days. These results

suggest that the three items were shown to emit ethylbenzene that could have some potential for increasing VOC levels in rooms where they are operated. However, the contribution to total indoor air is difficult to interpret in the absence of actual measurements.

The conclusion based on this review of available information is that building materials or furnishings are unlikely to be significant sources of ethylbenzene in indoor air compared to automotive sources. While only limited information on emissions from styrenic consumer electronic devices is available, it is expected that they would provide minimal contribution and that their contribution would be captured by the indoor air levels discussed in the following section.

6.3.1.4 Environmental Tobacco Smoke

Ethylbenzene and other aromatic hydrocarbons, such as benzene, styrene, and xylenes have been measured in cigarette smoke (Pankow *et al.*, 2004), and some biomonitoring data indicate that smoking is an important determinant of human exposure to ethylbenzene (see Section 6.0).

Wallace and Pellizzari (1986) reported significantly higher overnight indoor air ethylbenzene concentrations during the fall and winter in homes with smokers ($8.3 \mu\text{g}/\text{m}^3$, $n = 345$) compared to homes without smokers ($5.1 \mu\text{g}/\text{m}^3$, $n = 164$) (a ratio of 1.6). This difference was not observed during the spring and summer when homes were more open. Data from the TEAM studies have been analyzed using positive matrix factorization in order to elucidate sources (Anderson *et al.*, 2001). Ethylbenzene and other aromatics were determined to be associated with ETS (as well as gasoline vapors and automobile exhaust) and ambient concentrations.

Kim *et al.* (2001) also reported significant differences in ethylbenzene concentrations in smoking and non-smoking homes in Birmingham, U.K. ($1.9 \pm 1.2 \mu\text{g}/\text{m}^3$ in six smoking homes vs. $2.7 \pm 1.2 \mu\text{g}/\text{m}^3$ in six non-smoking homes, a ratio of 1.4). In a subsequent study, personal exposure of 12 urban dwellers to ethylbenzene increased by a factor of 1.6 (a statistically significant difference) by exposure to ETS (Kim *et al.* [2002]).

However, other studies indicate that the influence of smoking on indoor air levels of ethylbenzene is not significant. Heavner *et al.* (1995) measured ethylbenzene concentrations using personal air samplers worn by 49 women living in Columbus, Ohio. Twenty-four of the women were married to non-smokers and 25 were married to smokers. Personal air samples were collected over a three-hour period on a February evening in 1991. The average ethylbenzene concentration in homes with a smoker ($3.07 \mu\text{g}/\text{m}^3$; $n = 25$) was slightly lower than but not significantly different than the average concentration of $3.35 \mu\text{g}/\text{m}^3$ ($n = 24$) detected in non-smoker homes. The fact that ethylbenzene was significantly elevated in homes where gasoline was stored suggests that any influence of smoking could have been overwhelmed by the stronger petroleum-related source (Heavner *et al.*, 1995).

In a similar study involving non-smoking women living and working in the greater Philadelphia area, ethylbenzene concentrations detected using personal air samplers were not significantly different for women married to a smoking spouse and women married to a non-smoking spouse (Heavner *et al.*, 1996). Workplace exposures were also measured. The authors found no difference between ethylbenzene concentrations measured for women

working in a smoking environment and women working in a nonsmoking environment (Table 6-18). The authors concluded, based on 3-ethenylpyridine/ethylbenzene ratios, that only 3.4% of personal exposure to ethylbenzene in the home, and 2.7% of personal exposure to ethylbenzene in the workplace, was attributable to ETS.

Table 6-18. Personal Air Ethylbenzene Concentrations Detected in Smoking and Nonsmoking Environments ^a

Study Location	Home/Work Type	Ethylbenzene Concentration ($\mu\text{g}/\text{m}^3$)					
		# Samples	Mean	Std. Dev.	Min.	Median	Max.
Ohio	Smoking Home	25	3.07	3.57	0.82	2.21	19.45
	Non-smoking Home	24	3.35	4.87	0.86	2.12	25.39
New Jersey/ Pennsylvania	Smoking Home	32	3.63	1.99	0.42	3.40	10.46
	Non-smoking Home	61	4.79	4.90	1.10	3.65	25.94
New Jersey/ Pennsylvania	Smoking Work	29	6.19	6.07	0.75	4.18	23.42
	Non-smoking Work	51	5.87	9.75	0.25	3.43	66.26

^a Source: Heavner *et al.* (1996)

Xie *et al.* (2003) examined the influence of ETS on concentrations of ethylbenzene and other VOCs in a test room. Factor and correlation analyses showed that while ethylbenzene concentrations in the room rose with smoking, there were clearly other sources present. The authors characterized these other sources as traffic exhaust, as well as indoor coating, emulsion varnish, furnishing, adhesives, and solvent used to decorate painted walls.

6.3.2 Ethylbenzene Levels in Non-Residential Buildings

6.3.2.1 Industrial Workplace

According to the National Occupational Exposure Study (NOES) conducted by National Institute for Occupational Safety and Health (NIOSH) from 1981 to 1983, an estimated 201,838 workers were potentially exposed to ethylbenzene in the workplace (NIOSH, 1991) (Table 6-19). Almost all of these workers are included in the refinery chain of commerce, not the ethylbenzene/styrene chain of commerce (only 2% of the total employees and less than 1% of female employees based upon SIC code 28). The NOES database does not contain information on the frequency, concentration, or duration of occupational exposure to any of the chemicals listed; it provides only estimates of the numbers of workers who had potential exposure in the workplace in the early 1980s.

Table 6-19. National Occupational Exposure Survey (1981 – 1983). Estimated Numbers of Employees Potentially Exposed to Ethylbenzene by 2-Digit Standard Industrial Classification (SIC) ^a

SIC	Industry Description (1972)	Total No. of Employees	Total No. of Female Employees
7	Agricultural Services	8,847	
13	Oil And Gas Extraction	2,227	
15	General Building Contractors	9,394	5
16	Heavy Construction Contractors	1,650	
17	Special Trade Contractors	29,137	
20	Food And Kindred Products	452	10
22	Textile Mill Products	81	81
23	Apparel And Other Textile Products	328	328
24	Lumber And Wood Products	14,176	4,148
25	Furniture And Fixtures	1,623	
26	Paper And Allied Products	771	
27	Printing And Publishing	16,963	2,367
28	Chemicals And Allied Products	3,903	306
29	Petroleum And Coal Products	11,053	319
32	Stone, Clay, And Glass Products	835	
33	Primary Metal Industries	39	
34	Fabricated Metal Products	27,143	17,791
35	Machinery, Except Electrical	7,476	479
36	Electric And Electronic Equipment	2,375	291
37	Transportation Equipment	10,745	1,259
38	Instruments And Related Products	1,429	814
39	Miscellaneous Manufacturing Industries	6,119	2,203
41	Local And Interurban Passenger Transit	670	
42	Trucking And Warehousing	100	
45	Transportation By Air	11,969	114
47	Transportation Services	86	
48	Communication	332	
49	Electric, Gas, And Sanitary Services	8,699	
50	Wholesale Trade - Durable Goods	2,807	
55	Automotive Dealers & Service Stations	1,723	
72	Personal Services	1,042	
73	Business Services	3,648	309
75	Auto Repair, Services, And Garages	8,386	
76	Miscellaneous Repair Services	460	
80	Health Services	4,962	3,490
84	Museums, Botanical, Zoological Gardens	184	92
TOTAL		201,833	34,405

^a Source: NIOSH (1991)

Several studies conducted in the 1980s indicated occupational ethylbenzene exposure levels in the tens of parts per billion. The average air concentrations of ethylbenzene measured over the full work shift for gasoline service station attendants, transport drivers, and outdoor refinery personnel were comparable at 63, 79, and 79 $\mu\text{g}/\text{m}^3$, respectively, during the summer of 1984 (Rappaport *et al.*, 1987). Not surprisingly, the authors noted that exposures of

service station attendants were significantly lower when vapor recovery systems were present.

According to the OECD SIDS for ethylbenzene (OECD, 2005), the processes for making ethylbenzene and styrene take place in a closed system, minimizing the potential for worker exposure. In particular, direct dermal contact is unlikely to occur. A survey of U.S. manufacturers of ethylbenzene conducted by the Styrene and Ethylbenzene Association (SEBA), referred to in the ATSDR toxicological profile as a written communication dated 1990, indicated that typical workplace exposure levels of ethylbenzene in styrene and/or ethylbenzene processing plants were in the range of 0.1-1 ppm (433 – 4,333 $\mu\text{g}/\text{m}^3$) for an 8-hour time-weighted average (TWA) (Helmes, 1990, as cited in ATSDR, 1999).

Seven U.S. ethylbenzene producers recently compiled worker exposure data collected between 1990 and 2000 (American Chemistry Council, 2001; Appendix J) There were a total of 1,727 personal monitoring samples (eight-hour, time-weighted averages) representing exposures for process operators, maintenance workers, loading/unloading, quality laboratory workers and supervisory/professional workers. As shown in Table 6-20, approximately 71% of the measurements were either non-detectable or less than 0.1 ppm (434 $\mu\text{g}/\text{m}^3$), 25% were between 0.1 ppm to 1.0 ppm (4,343 $\mu\text{g}/\text{m}^3$), 4% were greater than 1.0 ppm and less than 5 ppm (21,714 $\mu\text{g}/\text{m}^3$), and 0.3% were greater than 5 ppm. Of the six samples with concentrations greater than 5 ppm, four were less than 9 ppm (39,085 $\mu\text{g}/\text{m}^3$), and the other two results were unspecified. All of these values are very low compared to the chronic occupational exposure standard for ethylbenzene of 100 ppm (433,400 $\mu\text{g}/\text{m}^3$) adopted by several regulatory bodies (ICSC 0268; <http://www.cdc.gov/niosh/ipcsneng/neng0268.html>).

Table 6-20. Percent of 8-Hour Time-Weighted Average Personal Air Samples Containing Ethylbenzene in Exposure Ranges in Styrene Plants (ppm) ^a

Job Description	Non-detectable	<0.1 ^b	>0.1-1.0 ^c	>1.0-5.0 ^d	>5.0 ^e
Process Operator	51.6%	19.7%	25.0%	3.3%	0.4%
Maintenance	53.0%	15.9%	26.4%	4.2%	0.4%
Loading/Unloading	75.4%	10.8%	10.8%	3.1%	0.0%
Quality Laboratory	56.5%	9.6%	26.1%	7.8%	0.0%
Supervisory/Professional	16.7%	66.7%	16.7%	0.0%	0.0%
Total	53.1%	17.8%	24.9%	3.8%	0.3%

^a Source: American Chemistry Council (2001) (Appendix J)
^b < 433 $\mu\text{g}/\text{m}^3$
^c > 433 – 4,343 $\mu\text{g}/\text{m}^3$
^d > 4,343 – 21,714 $\mu\text{g}/\text{m}^3$
^e > 21,714 $\mu\text{g}/\text{m}^3$

Occupational exposure to ethylbenzene may also occur via inhalation at municipal waste composting facilities, where one study measured average air concentrations ranging from 600 – 38,100 $\mu\text{g}/\text{m}^3$ (0.14 - 7.3 ppm) (Eitzer, 1995). These levels are also very low compared to the occupational standard.

6.3.2.2 Office Buildings

Girman *et al.* (1999) measured concentrations of VOCs in indoor and outdoor air at 56 randomly selected public and private office buildings across the U.S. between the summer of 1995 and the winter of 1997 – 1998, as part of the EPA’s Building Assessment Survey and Evaluation (BASE) study. Ethylbenzene was one of eight VOCs measured in 81 – 99% of samples, with concentrations ranging from 0.3 – 30 $\mu\text{g}/\text{m}^3$.

Ethylbenzene concentrations were detected in 12 city and county office buildings located in the San Francisco Bay area, where smoking was prohibited except in designated areas (Daisey *et al.*, 1994). Samples were collected between June and September 1990. Six of the buildings had sealed windows and air conditioning, and six of the buildings were naturally ventilated. Indoor air ethylbenzene levels ranged from 0.3 to 1 $\mu\text{g}/\text{m}^3$ with a geometric mean of 0.5 $\mu\text{g}/\text{m}^3$. The I/O ratios for the buildings ranged from 0.48 to 2.5. The authors concluded that the probable source was motor vehicle exhaust.

Shields *et al.* (1996) compared geometric mean ethylbenzene concentrations in 50 sparsely populated telecommunications (Telco) offices, 11 densely occupied administrative (Admin) offices, and nine variably occupied data centers (Data) located throughout the country. Very little smoking took place in any of these buildings. Occupant densities per 1,000 ft^2 were 0.1 to 4 for Data, less than 0.4 occupants for Telco, and 3 – 5 for Admin. Ventilation rates were comparable in the Telco and Admin offices, and both were better ventilated than the data centers. Ethylbenzene was detected in all samples from all buildings, and in 94% of the outdoor samples. Results are shown in Table 6-21.

Table 6-21. Detection Frequency and Geometric Mean Concentrations and Indoor/Outdoor Ratios of Ethylbenzene in Three Types of Office Buildings^a

Location	Detection Frequency	Concentration in Air ($\mu\text{g}/\text{m}^3 \pm \text{GSD}$)	Indoor/Outdoor Ratio
Telco	100%	1.6 \pm 2.0	1.6 \pm 2.3
Admin	100%	2.0 \pm 1.9	1.9 \pm 1.8
Data	100%	2.7 \pm 1.9	2.7 \pm 1.6
Outdoor	94%	1.0 \pm 1.6	--

^a Source: Shields *et al.* (1996)

As indicated in Table 6-21, ethylbenzene concentrations varied little with either occupancy or ventilation. The geometric mean I/O ratios were similar for the Telco and Admin offices (which had comparable ventilation rates), but somewhat higher in the less-ventilated Data offices. The authors interpreted the difference between the Telco and Admin offices as having to do with a larger contribution from indoor sources in the Admin offices when comparable ventilated facilities are compared (Shields *et al.*, 1996).

Hodgson *et al.* (1996) found that tobacco smoke contributed to the ethylbenzene detected in designated smoking areas in office buildings. Ethylbenzene concentrations were measured in designated smoking areas in five different office buildings. Concentrations ranged from 1.3 to 8.7 $\mu\text{g}/\text{m}^3$ (over a five-hour sampling time). Using 3-ethenylpyridine as a tracer for ETS,

the authors concluded that ETS contributed about 20% of the indoor air ethylbenzene concentrations.

6.3.2.3 Schools

Several recent studies have focused on children’s exposures to VOCs in school buildings. Exposures to children between 7 and 13 years of age attending two inner-city schools in Minneapolis, Minnesota were estimated by recording VOC concentrations in concurrent samples of indoor air at home and at school, in outdoor air, and in personal air (Adgate *et al.*, 2004). Samples were collected in winter (January-February) and spring (April-May), 2000. Personal air samples and indoor air samples at home were collected over a 48-hour period. Indoor air samples at school were collected only during school hours for a period of one week – on average, about 31 hours over five days. Outdoor air samples were collected continuously, on school grounds, between Monday morning and Friday afternoon (about 103 hours). Ethylbenzene concentrations are reported in Table 6-22. There was little seasonal variation in ethylbenzene concentrations in measurements in homes, outdoors or personal monitors; however, median concentrations in schools were 50% lower in the spring than in the winter measurements likely the result of open windows in the spring.

Table 6-22. Ethylbenzene Concentration Detected in Minneapolis, Minnesota School Buildings Compared to Other Locations ^a

Season	Sample Type	Ethylbenzene Concentration ($\mu\text{g}/\text{m}^3$)		
		10 th Percentile	Median	90 th Percentile
Winter	Home	0.6	1.0	2.8
	School	0.2	0.6	1.0
	Outdoor	0.2	0.6	0.8
	Personal	0.6	1.0	2.4
Spring	Home	0.5	1.0	3.8
	School	0.2	0.3	0.5
	Outdoor	0.3	0.5	0.7
	Personal	0.5	0.9	2.0

^a Source: Adgate *et al.* (2004)

Similar ethylbenzene concentrations were recorded in portable and traditional classrooms in Los Angeles County (Shendell *et al.*, 2004). Integrated daily average ethylbenzene concentrations detected during the school day, over a period of one week, ranged from 1.0 to 1.5 $\mu\text{g}/\text{m}^3$ in portable classrooms and 0.1 to 1.0 $\mu\text{g}/\text{m}^3$ in traditional classrooms (samples collected in June, 2000). Concentrations of ethylbenzene in samples collected during February/March 2001 were slightly higher, ranging from 1.1 to 2.6 $\mu\text{g}/\text{m}^3$ in portable classrooms and from 0.8 to 2.5 $\mu\text{g}/\text{m}^3$ in traditional classrooms. These data suggest that exposures to ethylbenzene in school environments are similar to exposure levels in outdoor air in this urban area.

6.3.3 Motor Vehicles

In a study designed to assess the effects of traffic patterns, driving periods, and ventilation conditions on in-vehicle VOC concentrations, Chan *et al.* (1991) measured one-hour average ethylbenzene concentrations in two different vehicles over three different roadways (n = 77). The concentrations ranged from 0.8 to 21.8 $\mu\text{g}/\text{m}^3$, with a mean of 8.8 $\mu\text{g}/\text{m}^3$. Ethylbenzene levels in ten ambient air samples along the driver's route ranged from 5.2 to 12.1 $\mu\text{g}/\text{m}^3$, with an average of 9.2 $\mu\text{g}/\text{m}^3$. In comparison, sidewalk measurements (n = 44) were lower, ranging from 4.9 to 7.8 $\mu\text{g}/\text{m}^3$ with an average value of 5.9 $\mu\text{g}/\text{m}^3$. The I/O ratio based on the mean values in the cars vs. the sidewalk was 1.5. The highest in-vehicle concentrations were detected under urban driving conditions (median = 11.3 $\mu\text{g}/\text{m}^3$, n = 34), while rural conditions yielded the lowest in-vehicle concentrations (median = 1.2 $\mu\text{g}/\text{m}^3$; n = 8). The median ethylbenzene concentration recorded while driving on the highway was 6.5 $\mu\text{g}/\text{m}^3$ (n = 35). There were no significant differences in ethylbenzene concentrations between vehicles, but the authors found lower concentrations when air conditioning was used compared to conditions with windows open or windows closed with vent open and fan on (Chan *et al.*, 1991).

Weisel *et al.* (1992) showed that ethylbenzene and other gasoline VOCs are at higher concentrations in vehicle air than in ambient air during idling and under suburban and urban driving conditions. Ethylbenzene concentrations recorded during a suburban commute into New York City averaged 3.3 $\mu\text{g}/\text{m}^3$ under high ventilation conditions and 4.4 $\mu\text{g}/\text{m}^3$ under low ventilation conditions. Average concentrations were higher when traveling on the New Jersey turnpike (7.9 $\mu\text{g}/\text{m}^3$) and in the Lincoln Tunnel (10 $\mu\text{g}/\text{m}^3$). Lower levels of ethylbenzene, ranging from 0.58 to 1.6 $\mu\text{g}/\text{m}^3$, were recorded in a closed vehicle after idling for 30 minutes.

In a similar study, the median in-vehicle ethylbenzene concentrations measured on 113 commutes through suburban New Jersey and 33 New Jersey/New York commutes over a 19-month period was 6.8 $\mu\text{g}/\text{m}^3$ (Lawryk and Weisel, 1996). The cars used in this study were a 1988 Chevrolet Celebrity and a 1987 Plymouth Horizon; concentrations were consistently higher in the Horizon. The sample collection time on an average commute was 45 minutes. Average concentrations were somewhat higher under low-ventilation conditions (11.5 $\mu\text{g}/\text{m}^3$) compared to high-ventilation conditions (8.5 $\mu\text{g}/\text{m}^3$). In addition, ethylbenzene concentrations detected in commutes involving travel through the Lincoln Tunnel averaged 14.3 $\mu\text{g}/\text{m}^3$, significantly higher than those measured on the suburban commute (8.5 $\mu\text{g}/\text{m}^3$) and during turnpike driving (8.8 $\mu\text{g}/\text{m}^3$). Concentrations in the Celebrity were not higher than those in ambient air, but those in the Horizon were. The average I/O ratio of the two cars (estimated from Figure 3 in the publication) is around 1.7.

Ethylbenzene concentrations measured in buses traveling through residential, commercial, and heavily industrialized areas in Detroit, Michigan averaged 1.1 $\mu\text{g}/\text{m}^3$ during morning routes and 2.2 $\mu\text{g}/\text{m}^3$ during afternoon routes, and were similar to outdoor air concentrations (Batterman *et al.*, 2002).

Reidiker *et al.* (2003) evaluated exposure of ten non-smoking Wake County, North Carolina State Highway Patrol officers to ethylbenzene and other air pollutants inside patrol cars (1998 – 2000 Ford Crown Victorias), a fixed ambient location, and changing roadside

locations. In-vehicle ethylbenzene concentrations (~10-hour averages) ranged from 1.3 – 11.3 $\mu\text{g}/\text{m}^3$ (mean = 3.9 $\mu\text{g}/\text{m}^3$) over 50 patrol shifts, while means at the ambient and roadside locations were both 0.9 $\mu\text{g}/\text{m}^3$ (I/O = 1.4 to 12.2). The elevated in-vehicle concentrations were attributed to gasoline.

Fedoruk and Kerger (2003) compared in-vehicle concentrations of ethylbenzene and other VOCs in several car models (new [1997] and used [1993]) under different static (parked, unventilated) and driving under various ventilation conditions in the Los Angeles, California area and Foxboro, Massachusetts area. Unlike earlier studies, this study examined the effects of temperature on the concentration and composition of VOCs. Total VOC concentrations were four to eight times higher under high-heat (118 – 145 °F) than moderate-heat (90 – 109 °F) conditions in the cars, and tended to be higher in new than used cars. The authors remarked that "...prominent concentrations of styrene and phenol...are presumed [to be] off-gassing products from vehicle interior components" (Fedoruk and Kerger, 2003). The fact that: (1) ethylbenzene was not among the top ten compounds measured under any conditions; and, (2) the fold-increase in ethylbenzene concentrations under high- vs. moderate-heat conditions was similar to that of other gasoline components suggest that any ethylbenzene release from styrenic materials in cars was insignificant. Ethylbenzene concentrations averaged 4.2 $\mu\text{g}/\text{m}^3$ (range = 2.5 to 7.5 $\mu\text{g}/\text{m}^3$) under moderate-temperature static conditions, and fell to 2 $\mu\text{g}/\text{m}^3$ during driving. The authors suggested that the lower levels recorded in this study (compared to a median concentration of 11.3 $\mu\text{g}/\text{m}^3$ recorded by Chan *et al.* (1991) may be attributed to the use of reformulated gasoline in California.

6.3.4 Summary

The foregoing review indicates that the dominant source of ethylbenzene in indoor air, as in outdoor air, is motor vehicle emissions. In urban environments, the contribution from these sources evidently obscures that from indoor sources, including ETS, household chemicals, and possibly building materials and furnishings and household electronic devices. As with outdoor levels of ethylbenzene, indoor levels have decreased in recent years.

Although a fairly large data set of both indoor air and outdoor concentrations of VOCs are available, these data may not be gathered at the same time or in the same geographic area making a comparison between the two data sets (*e.g.*, indoor and outdoor) difficult. Further, because the available studies differ significantly in their purposes, study designs, methods for collecting and analyzing samples, and data analysis and reporting, the comparability of their absolute results is questionable. Given these limitations, the available data were not considered adequate for exposure assessment. Rather, the approach that was taken was based on: (1) the relatively rich and high-quality data set available for ambient air; and, (2) published or calculated estimates of I/O ratios in different microenvironments. That is, ambient air concentrations were multiplied by microenvironment-specific I/O ratios to estimate ethylbenzene levels typically encountered in these locations. This approach takes advantage of the robustness of the outdoor air data and avoids data quality and comparability issues in the indoor studies.

Ethylbenzene exposure concentrations calculated in this manner are summarized in Table 6-23, and discussed in the following sections.

Table 6-23. Summary of Microenvironment-Specific Ethylbenzene Exposure Concentrations in Air ($\mu\text{g}/\text{m}^3$)

Setting	Urban		Rural/Suburban	
Microenvironment	Smoking ^b	Non-Smoking	Smoking ^b	Non-Smoking
Home^a	3.1	3.1	3.1	3.1
Central tendency	5.1E+00	3.4E+00	2.7E+00	1.8E+00
Upper bound	1.1E+01	7.5E+00	7.2E+00	4.8E+00
Office^a	1.5	1.5	2.1	2.1
Central tendency	2.4E+00	1.6E+00	1.8E+00	1.2E+00
Upper bound	5.4E+00	3.6E+00	4.8E+00	3.2E+00
School^a	1.5	1.5	2.1	2.1
Central tendency	--	1.6E+00	--	1.2E+00
Upper bound	--	3.6E+00	--	3.2E+00
Motor Vehicle^a	4.2	4.2	4.2	4.2
Central tendency	6.9E+00	4.6E+00	3.7E+00	2.5E+00
Upper bound	1.5E+01	1.0E+01	9.7E+00	6.5E+00

^a – Values in these rows are representative of the I/O ratios used.
^b – Ethylbenzene concentrations in indoor air in smoking homes was determined by multiplying the non-smoking concentration by a factor of 1.5.

6.3.4.1 Homes

As discussed previously, ethylbenzene levels in urban buildings are primarily determined by outdoor sources, most notably motor vehicle emissions. I/O ratios in the urban studies discussed in Section 6.3.1 were 1.2 (Ilgen *et al.*, 2001b), 5 (Van Winkle and Scheff, 2001), 1.8 (from Kinney *et al.*, 2002 for New York), 1.1 (Sax *et al.*, 2004), 2.6 (Payne-Sturges *et al.*, 2004) and 5.6 (Sexton *et al.*, 2004). The average of these six values is 2.9; however, the inclusion of the Ilgen *et al.* (2001b) is questionable since it is a non-U.S. study. Excluding this study results in an I/O ratio of 3.2 considering the other studies. A value of 3.1, approximately half way between, and within approximately $\pm 5\%$ of, the two calculated I/O ratios was selected as the I/O ratio for use in this assessment. This value was selected over EPA’s default I/O ratio of 5 for ethylbenzene (EPA, 1998) because it is representative of measured values. Applying this ratio to the central tendency and upper-bound urban ambient air concentrations of 1.09 and 2.43 $\mu\text{g}/\text{m}^3$ (Section 6.2.3.1) yielded corresponding urban home concentrations of 3.4 and 7.5 $\mu\text{g}/\text{m}^3$.

The majority of recent U.S. indoor air studies have been conducted in cities. In the absence of values from U.S. studies in rural areas, the I/O ratio of 3.1 from urban areas was applied to the central tendency and upper-bound ambient air concentrations in rural/suburban areas of 0.59 and 1.54 $\mu\text{g}/\text{m}^3$ (Section 6.2.3.1), yielding corresponding rural/suburban home concentrations of 1.8 and 4.8 $\mu\text{g}/\text{m}^3$.

The available data suggest but do not unambiguously establish the importance of ETS as a source of ethylbenzene in indoor air. However, the weight of the evidence seems to indicate that smoking can increase both exposure concentrations and ethylbenzene levels in blood and breath (Sections 6.1.1 and 6.1.2). The data of Ashley *et al.* (1995) indicate a ratio of ethylbenzene concentrations in the blood of smokers and non-smokers of 1.1 (0.17/0.1), while those of Perbellini *et al.* (2002) indicate a ratio of 1.7 (0.243/0.222) (Table 6-4). These

values are similar to the ratios of air concentrations reported by Wallace and Pellizzari (1986) ($8.3/5.1 = 1.6$), Kim *et al.* (2001) ($2.7/1.2 = 1.4$), and Kim *et al.* (2002) (1.6). On this basis, the average of the three values derived from the air studies discussed in Section 6.3.1.4 (1.5) was applied to indoor air concentrations to estimate the additional exposure associated with ETS.

6.3.4.2 Industrial Workplace

The American Chemistry Council's (2001) (Appendix J) recent study of styrene manufacturing workers' exposures to ethylbenzene was considered the most appropriate for use in the exposure assessment. The majority (71%) of workers in this study were exposed to less than 0.1 ppm ($434 \mu\text{g}/\text{m}^3$); accordingly, this value was selected as the central tendency for Production Worker exposure. Approximately 96% of the workers were exposed to less than 1 ppm ($4,343 \mu\text{g}/\text{m}^3$), which was therefore selected as the upper-bound exposure estimate.

6.3.4.3 Office Buildings

The available data suggest that concentrations of ethylbenzene in office buildings may be lower than those typical of residences (Hodgson and Levin, 2003). Daisey *et al.* (1994) reported a range of I/O ratios for San Francisco Bay Area office buildings of 0.48 to 2.5 (Section 6.3.2.2); applying the average of these values, 1.5, to the central tendency and upper-bound ambient air estimates for urban environments results in estimated urban office building concentrations of 1.6 and $3.6 \mu\text{g}/\text{m}^3$.

Shields *et al.*'s (1996) study of various types of offices throughout the U.S. was considered more applicable to the rural/suburban setting. Application of the average in I/O ratios presented in Table 6-21 (2.1) results in estimated rural/suburban office building concentrations of 1.2 and $3.2 \mu\text{g}/\text{m}^3$.

Both smoking and non-smoking office microenvironments were considered, applying the ETS factor discussed in Section 6.3.1.4.

6.3.4.4 Schools

The very few data available for schools report indoor air VOC concentrations similar to those in outdoor air (Table 6-22). To avoid underestimation of exposure in this important microenvironment, however, the I/O factors identified for office buildings in the preceding section were also used for schools. Thus, indoor air concentrations in office buildings and schools were assumed to be the same. However, schools were assumed to be ETS-free.

6.3.4.5 Motor Vehicles

The studies examined suggest that older cars that lack catalytic converters (and may have fuel leakage problems) tend to have higher internal concentrations of ethylbenzene and other fuel-related VOCs than newer models. However, the available studies consistently suggested that the major determinant of in-vehicle ethylbenzene concentrations was ambient concentrations. Chan *et al.* (1991) observed an I/O ratio of in-vehicle to sidewalk concentrations of $8.8 \mu\text{g}/\text{m}^3/5.9 \mu\text{g}/\text{m}^3 = 1.5$ (Section 6.3.3). An I/O of 1.7 was estimated

from the data of Lawryk and Weisel (1996). An I/O range of 1.4 – 12.2 was estimated from the data of Reideker *et al.* (2003). Application of the average of these values, 4.2, results in central tendency and upper-bound values for the urban setting of 4.6 to 10 $\mu\text{g}/\text{m}^3$ (6.9 to 15 $\mu\text{g}/\text{m}^3$ with smoking), and 2.5 to 6.5 $\mu\text{g}/\text{m}^3$ (3.7 to 9.7 $\mu\text{g}/\text{m}^3$ with smoking) for the rural/suburban setting.

6.4 Ethylbenzene Levels in Food

Ethylbenzene does not appear to be naturally occurring in plants (Tang *et al.*, 2000), and as discussed in Section 5.2.3, bioaccumulation in the aquatic or terrestrial food chains is not expected to occur. As a result, levels in fresh (unpackaged) foods are generally very low. However, foods may be subject to potential accumulation of ethylbenzene by partitioning from ambient atmospheric sources, as well as migration from styrenic food packaging and contact materials. In this section, available measured data are reviewed and summarized, and models are used to estimate potential migration from food-contact materials. These data are used in Section 6.7.2.2 to calculate daily dietary ethylbenzene exposures.

As studies of ethylbenzene in fresh food are limited, both international and U.S. specific reports are presented in the following sections. The international data is presented to provide a more complete picture of ethylbenzene concentrations in fresh food, but U.S. data was preferential used in the estimation of intake.

6.4.1 Food Studies

Few published studies address ethylbenzene levels in food. In a review of styrene and ethylbenzene levels in foods, Tang *et al.* (2000) stated that “the presence of ethylbenzene in foods appears primarily as a result of migration from polymer packaging materials, mostly from polystyrene.” However, specific evidence was not presented. In samples of fresh foods, the concentration of ethylbenzene was generally very low (near the limit of detection, 0.1 $\mu\text{g}/\text{kg}$) in fruits and fruit products (tomatoes, apples, strawberries, kiwi fruit) and in some vegetables and vegetable seeds. Assuming a total dietary content of 5 – 20 $\mu\text{g}/\text{kg}$, a consumption rate of 1 kg food per day⁵, and applying the U.S. Food and Drug Administration (FDA) consumption factor of 0.1 (FDA, 2002), Tang *et al.* (2000) estimated that adults might consume 0.1 – 0.3 μg ethylbenzene /kg-day as a worst case.

The Volatile Compounds in Food database (VCF, 2005) reports the natural occurrence of volatile compounds in fresh and cooked food items. It lists 145 references for ethylbenzene (VCF 8.1, Appendix K). The few measured values presented in this database are listed in Table 6-24. The fact that many of these references are decades old and from foreign sources reduces their potential usefulness for this exposure assessment. However, it is noteworthy that, consistent with other food surveys, very low ethylbenzene levels were measured in fresh, raw foods such as uncooked beans, kiwifruit, and mountain papaya, while foods with a high fat content, such as butter and cheeses, had levels ranging from 7 $\mu\text{g}/\text{kg}$ in butter up to 241 $\mu\text{g}/\text{kg}$ in Gruyere cheese. The reason(s) for the relatively high concentrations reported in

⁵ 1 kg/day = estimated total consumption rate of meat, fish, dairy products, eggs, vegetables, butter, margarine, and oils (Tang *et al.* 2000).

two South American fruits (loquat and curuba) (Fröhlich *et al.*, 1989, Fröhlich and Schreier, 1990) are not known, but they are unlikely to be pertinent to American consumers. The data for grapes and fermented tea are also of uncertain relevance as they derive, respectively, from a 30-year old Russian publication and a 20-year old Chinese publication (see Appendix K).

Table 6-24. Concentrations ($\mu\text{g}/\text{kg}$) of Ethylbenzene in Foods from the Volatile Compounds in Food (VCF) Database

Food Item	Ethylbenzene Concentration ($\mu\text{g}/\text{kg}$)
Animal Products	
Butter	7
Cheese, Gruyere de Comte	4 - 241
Chicken, roasted	30
Egg, boiled	2 - 4
Egg, scrambled	4
Shrimp (cooked)	1
Skim milk, powder	1.9
Vegetable Products	
Beans, raw	1
Dill herb	70 - 150
Kiwifruit	1
Loquat	10 - 100
Mountain papaya	<10
Curuba (banana passion fruit)	10 - 100
Peas	4
Tea, partially fermented	0 - 700
Grapes (<i>Vitis vinifera</i> L.)	70 - 110

Several more recent food surveys provide current information regarding typical concentrations of ethylbenzene in foods. In 1993, the Ministry of Agriculture Fisheries and Food (MAFF) performed a survey of ethylbenzene and other aromatic hydrocarbons as part of the Total Diet Study in the United Kingdom (UK) (MAFF, 1995). Samples of 20 food groups collected from ten locations in the U.K. were analyzed. Ethylbenzene was not detected above the limit of detection ($2 \mu\text{g}/\text{kg}$) in most food group samples, although it (and several other compounds) were detected in most samples of carcass meat, offal, meat products, poultry, fish, and nuts (Table 6-25). A range of daily intakes of $0.3 - 4.2 \mu\text{g}/\text{day}$ was estimated by MAFF by calculating exposures under two assumptions: (1) non-detected values indicated absence of ethylbenzene, and (2) non-detected values indicated presence of ethylbenzene at the detection limit.

In a study published in 1995, Heikes *et al.* (1995) measured the concentration of 45 VOCs in 234 foods that are included in the Total Diet Study (TDS). Ethylbenzene was detected in only 15 of these products (6%) at an average concentration of $14.6 \mu\text{g}/\text{kg}$ (range = 6.37 to $38.7 \mu\text{g}/\text{kg}$). The four food items with the highest ethylbenzene concentrations were margarine ($38.7 \mu\text{g}/\text{kg}$), cake doughnuts ($23.9 \mu\text{g}/\text{kg}$), butter ($17.1 \mu\text{g}/\text{kg}$) and sandwich cookies ($15.1 \mu\text{g}/\text{kg}$).

Table 6-25. Concentrations (µg/kg) of Ethylbenzene in Ten Samples of Each Food Group from the 1993 U.K. Total Diet Study ^a

Food Group	Ethylbenzene Concentration (µg/kg)	
	Range	Mean
<i>Animal Products</i>		
Carcass meat	BDL – 3	BDL
Eggs	BDL	BDL
Fish	BDL – 5	4
Meat products	BDL - 10	4
Milk	BDL	BDL
Milk products	BDL	BDL
Offal	BDL – 5	3
Oils & fats	BDL	BDL
Poultry	BDL – 6	4
<i>Fruits/Vegetables</i>		
Canned vegetables	BDL	BDL
Green vegetables	BDL	BDL
Potatoes	BDL	BDL
Other vegetables	BDL	BDL
Fruit	BDL	BDL
Fruit products	BDL	BDL
Nuts	BDL - 38	7
<i>Grains</i>		
Bread	BDL	BDL
Other cereals	BDL – 2	BDL
<i>Other</i>		
Beverages	BDL	BDL
Sugars	BDL – 2	BDL
^a Source: MAFF (1995) BDL = below detection limit (2 µg/kg)		

The TDS (or Market Basket Study) is an ongoing FDA program that determines levels of various nutrients and chemicals (including ethylbenzene) in table-ready⁶ foods representing the major components of the U.S. diet (FDA, 2004a). Coupled with age group-specific estimates of the daily consumption rates of each food, measured analyte concentrations are used to calculate dietary intakes for the U.S. population. In order to conduct a more detailed evaluation of ethylbenzene concentrations in the U.S. diet, analytical results from 1998 – 2001 (the most recent year available on-line), representing 15 seasonal market basket sampling events (four per year for each year except 1999, which had three events), were combined and sorted by chemical and food type. Ethylbenzene was detected at least once in 65 out of 320 items analyzed for VOCs (20%). Minimum, maximum, and arithmetic mean ethylbenzene concentrations were determined for the 47 items in which the compound was detected at least three times in the 15 sampling events (Table 6-26). Of these 379 total samples, results for the majority (219/379 = 58%) were labeled “trace,” indicating a concentration greater than or equal to the limit of detection but less than the limit of

⁶ “Table-ready” indicates items purchased from a supermarket and prepared for consumption as they would be in a domestic kitchen.

quantitation. This suggests that less than 10% of food items consumed in the U.S. may contain detectable levels of ethylbenzene. As shown in Table 6-26, ethylbenzene concentrations ranged from 2 (“trace”) to 224 µg/kg (detected in blueberry muffins). Relatively high levels were also observed in other “grain” items. Average concentrations ranged from 2.3 to 50 µg/kg. Foods commonly eaten by infants and children (infant formulas, milk, and the only fresh fruit on the list, raw apple) were among the 15 foods with highest concentrations.

Levels of VOCs in twenty samples of 70 table-ready foods were measured over a five-year period (1996 to 2000) (Fleming-Jones and Smith, 2003). VOCs were detected in all foods, but analytical results were only reported for the 41 that had at least one of the 27 detected VOCs present at a concentration of 100 µg/kg or greater. Foods exclusively consumed by young children (milk- and soy-based infant formulas, baby foods and juices) were not among those in this category.

Ethylbenzene was detected in 28 of the 41 (68%) products reported (Table 6-27), with levels ranging from 2 µg/kg to a maximum of 101 µg/kg in blueberry muffins (which also contained relatively high levels of other compounds). Because ethylbenzene concentrations exceeded 100 µg/kg in only one item, only this item could theoretically owe its inclusion to ethylbenzene alone. Ethylbenzene was not detected in raw fruits (strawberries, oranges, bananas, avocados) or in processed foods that were not likely to have come in contact with polymer packaging materials (with the possible exception of canned tuna) (Fleming-Jones and Smith, 2003). This observation is consistent with Tang *et al.*'s (2000) observation that polystyrene food-contact materials appear to be a significant source of ethylbenzene in food. However, taken as a whole, the TDS data indicate that ethylbenzene is a minor contributor to VOC levels in the U.S. diet, including foods consumed in higher quantities by children.

Górna-Binkul *et al.* (1996) measured concentrations of ethylbenzene and other VOCs in a number of fruits (the pulp and peel of apples, kiwi fruit, pears, plums and the peel of oranges, lemons, grapefruit, mandarin oranges, grapes, and avocado) and vegetables (peel and pulp of a tomato; chicory, cabbage, Brussels sprouts, parsley and celery leaves; paprika peel; and bulb/root of turnips, potatoes, radishes, parsnips, and carrots) purchased in local shops in Toruń, Poland. Ethylbenzene was only found in orange peel (23.6 µg/kg dry weight) and parsley leaves (256.7 µg/kg dry weight). In general, VOC content was dependent on plant species and morphological part; about two-fold higher levels were found in the peel than the pulp, presumably due to the barrier function of this structure as well as its lipid content. None of the VOCs were detected in root vegetables, as would be expected given the physicochemical properties of VOCs. A subsequent study with toluene also showed accumulation of airborne compound in citrus peels, with little penetration into edible pulp (Ligor and Buszewski, 2003).

Table 6-26. Concentrations of Ethylbenzene in FDA Total Diet Study Food Items, 1998 - 2001^a

Category/Food Item	No. Detects	Ethylbenzene Concentration (µg/kg)		
		Minimum	Maximum	Mean
<i>Dairy</i>				
Cream cheese	3	25.0	100.0	50.0
Milk-based infant formula, low iron, ready-to-feed	9	25.0	50.0	30.6
Swiss cheese	4	25.0	50.0	37.5

Table 6-26. Concentrations of Ethylbenzene in FDA Total Diet Study Food Items, 1998 - 2001^a

Category/Food Item	No. Detects	Ethylbenzene Concentration (µg/kg)		
		Minimum	Maximum	Mean
Vanilla flavored light ice cream	7	25.0	50.0	28.6
Vanilla ice cream	3	25.0	50.0	41.7
Whole milk, fluid	14	25.0	50.0	30.4
Eggs				
Eggs, scrambled with added milk and fat	5	2.0	50.0	22.0
Fruits and Vegetables				
Soy-based infant formula, ready-to-feed	6	25.0	50.0	37.5
Apple, red with peel, raw	4	7.0	50.0	39.3
Meat				
Beef, ground, regular hamburger, cooked in patty shape	3	2.0	3.0	2.3
Bologna	6	2.0	4.0	3.0
Chicken nuggets, fast-food	12	2.0	50.0	8.0
Chicken, fried (breast, leg, and thigh), fast-food	6	2.0	50.0	14.7
Frankfurters, (beef/beef and pork), boiled	6	2.0	6.0	3.7
Meatloaf, beef, homemade	3	2.0	25.0	9.7
Pork, bacon, oven cooked	4	2.0	3.0	2.5
Quarter-pound cheeseburger on bun, fast-food	6	2.0	11.0	3.8
Quarter-pound hamburger sandwich on white roll with garnish, fast-food type	8	2.0	25.0	7.8
Salami, lunch meat type, regular, not hard	5	2.0	8.0	3.2
Taco/tostada, from Mexican carry-out	6	2.0	8.0	3.8
Fish				
Fish sticks, commercial, frozen, oven cooked	9	2.0	25.0	7.1
Tuna, canned in oil, drained	4	2.0	50.0	26.0
Fast food				
Cheese and pepperoni pizza, regular crust, from pizza carry-out	6	2.0	5.0	3.0
Cheese pizza, regular crust, from pizza carry-out	5	2.0	50.0	12.6
French fries, fast food	9	2.0	50.0	10.3
Potato chips, commercial	9	2.0	26.0	14.8
Fats				
Butter, regular (salted)	19	2.0	50.0	18.1
Margarine, stick, regular (salted)	15	2.0	50.0	13.6
Olive/safflower oil	14	2.0	50.0	20.7
Grains				
Apple pie fresh/frozen, commercial	8	2.0	10.0	4.8
Brownies, commercial	9	2.0	25.0	8.4
Butter-type crackers (e.g., Ritz, Hi-Ho)	5	3.0	100.0	28.2
Cake doughnuts with icing, any flavor, from doughnut store	10	2.0	8.0	4.9
Chocolate cake with chocolate icing, commercial	11	2.0	50.0	8.1
Chocolate chip cookies, commercial	8	2.0	100.0	29.0
Corn chips	3	2.0	3.0	2.3
Graham crackers	7	2.0	100.0	30.4
Muffins (blueberry/plain)	8	2.0	224.0	43.5
Popcorn, popped in oil	3	2.0	4.0	3.0
Sandwich cookies with cream filling, commercial	4	2.0	50.0	26.8
Sugar cookies, commercial	8	2.0	19.0	5.4
Sweet roll/Danish, commercial	6	2.0	5.0	3.3
White bread, enriched	8	14.0	100.0	33.4
Nuts				
Mixed nuts, no peanuts, dry roasted	16	3.0	38.0	12.8
Peanut butter, creamy, commercial in jar	12	2.0	50.0	9.3
Sweets				
Candy, caramels	5	2.0	100.0	31.2

Table 6-26. Concentrations of Ethylbenzene in FDA Total Diet Study Food Items, 1998 - 2001^a

Category/Food Item	No. Detects	Ethylbenzene Concentration (µg/kg)		
		Minimum	Maximum	Mean
Milk chocolate candy bar, plain	13	2.0	50.0	8.7

^a Source: FDA (2004a)

Table 6-27. Concentrations of Ethylbenzene (µg/kg) in FDA Total Diet Study Food Items Containing at Least 100 µg/kg of any VOC, 1996 - 2000^a

Category/Food Item	Detection Frequency ^b	Ethylbenzene Concentration (µg/kg)	
		Minimum	Maximum
Dairy			
American cheese	10%	3	4
Cheddar cheese	5%	12	12
Eggs			
Eggs, scrambled	5%	5	5
Meat			
Bologna	10%	2	4
Cheeseburger, quarter pound	15%	2	3
Chicken nuggets, fast food	25%	2	23
Frankfurters, beef	15%	3	4
Ground beef	5%	2	2
Pork bacon	5%	2	2
Quarter pound hamburger, cooked	15%	2	3
Fast food			
Cheese and pepperoni pizza	15%	2	3
Cheese pizza	5%	3	3
French fries, fast food	20%	2	5
Fats			
Butter	35%	7	14
Margarine	30%	3	11
Olive/safflower oil	10%	4	23
Grains			
Apple pie, fresh/frozen	25%	2	14
Blueberry muffin	20%	3	101
Cake doughnuts with icing	20%	3	14
Chocolate cake with icing	25%	2	13
Chocolate chip cookies	15%	2	11
Graham crackers	10%	6	23
Potato chips	10%	2	11
Sugar cookies	10%	2	5
Sweet roll/Danish	15%	2	5
Nuts			
Mixed nuts	35%	3	38
Peanut butter	30%	4	10
Sweets			
Fruit-flavored sherbet	5%	3	3

^a Source: (Fleming-Jones and Smith, 2003)
^b Computed as reported number of detects divided by 20 samples

6.4.2 Atmospheric Uptake Studies

Several studies have shown that ethylbenzene can enter food items grown in proximity to ethylbenzene sources or during processing operations, particularly when foods are processed or stored in small areas with poor ventilation. Prompted by a concern over benzene levels in olive oil, Biedermann *et al.* (1995, 1996) measured benzene, ethylbenzene, xylenes and toluene in the oil of olives freshly picked from trees and following several days of storage or milling for various time periods. The level of ethylbenzene detected in the oil of olives delivered for processing (6 - 8 µg/kg) increased three- to six-fold when the olives were stored in a room containing gasoline-powered vehicles, or with the amount of time milled (increased to 14, 20, 34, and 25 µg/kg when milled for 15, 30, 45, and 75 minutes, respectively). Processed extra virgin olive oil contained 11 to 27 µg/kg ethylbenzene, depending on the processing method. The measured concentrations in olive oil were larger than expected given the ambient air concentrations. The authors suggested that gasoline-powered engines used to transport and process olives were largely responsible for the amount of VOCs in the oil.

A study conducted by the U.K. MAFF contrasted levels of ethylbenzene and other VOCs from samples of fatty foods obtained near gasoline/automotive exhaust sources (petrol stations, busy roads) with those from shops at a distance from obvious sources (MAFF, 1996) (Table 6-28). Toluene was the compound most frequently detected; ethylbenzene was measurable in only a few items (reporting limit = 10 µg/kg). Ethylbenzene levels ranged from 9 to 12 µg/kg in butter, cheese, margarine and lard, and up to 20 µg/kg in sausage. Although the low detection rate for ethylbenzene precluded statistical analysis of the data, the results seem to indicate that shop location generally did not cause discernible differences in the concentrations of ethylbenzene in these foods. Indeed, the same conclusion was reached for toluene, the only compound with sufficient detections for statistical analysis. The apparent lack of agreement of these results with those of Biedermann *et al.* (1995, 1996) suggests that another (common) source of VOC exposure at some earlier stage in their production may have been a more important determinant of the observed VOC concentrations in these items than local traffic at retail locations.

Table 6-28. Summary of Overall Concentrations of Ethylbenzene (µg/kg) in Butter, Cheese, Lard, Margarine, and Sausage from Different Types of Shops ^a

Food Item	Shop Type	Mean Overall Ethylbenzene Concentration (µg/kg Whole Food)
Butter and Cheese	Near petrol station	9
Lard and Margarine		9
Butter and Cheese	Near busy road	12
Lard and Margarine		11
Sausage		19-20
Butter and Cheese	Low concentration area	9

^a Source: MAFF (1996)

6.4.3 Migration of Ethylbenzene from Styrenic Food-Contact Materials

As discussed in Section 5.1.2, the major use of ethylbenzene is in the production of styrene, the monomer used in the production of a wide variety of polystyrene-based products. Polystyrene containers (both GPPS and HIPS) are used as packaging for numerous foods and food products, egg cartons, water, milk, oils, hot and cold beverages, dairy products, desserts, and meat products. Polystyrene foam is used in cups, containers, single-service and hinged containers, and food service and other foam trays (Lickly *et al.*, 1995). GPPS and HIPS disposables products include tumblers, cocktail glasses, vending cups, dishes, plates, bowls, lids, closures and flatware (Lickly *et al.*, 1995).

All of these materials may contain residual levels of ethylbenzene (among other potential migrants). Migration of ethylbenzene and other chemicals from styrenic (and other) food-contact materials is a recognized means of chemical entry into packaged foods and the subject of national and international regulation (*e.g.*, FDA, 2002; Arvanitoyannis and Bosnea, 2004; Begley *et al.*, 2005; Mercea, 2005). Migration is a diffusion process that may be strongly influenced by the interaction of food components with the packaging material (Figure 6-5). For example, fat can migrate into plastic, increasing the mobility of plastic components and enhancing their migration into food.

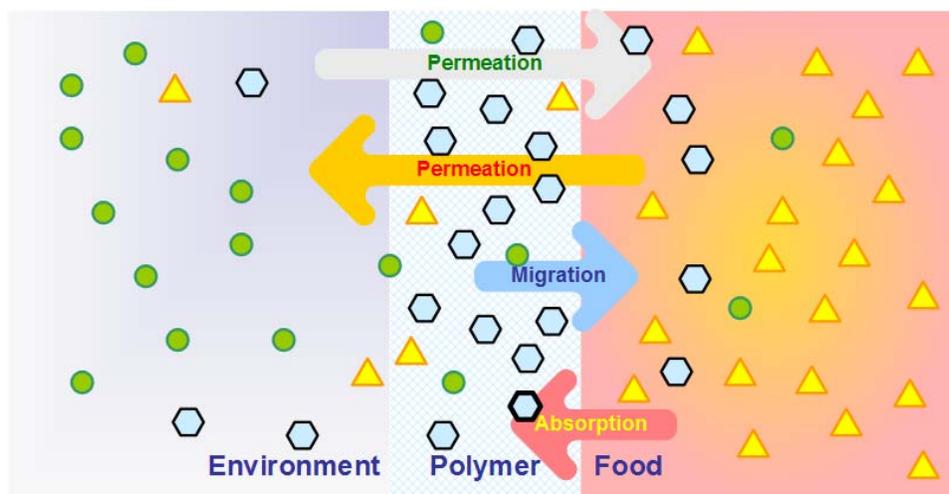


Figure 6-5. Polymer-Food Interface

Estimated concentrations of ethylbenzene in GPPS and HIPS packaging materials and disposables presented in a report prepared by the Polystyrene Work Group (PSWG) of the Food, Drug, and Cosmetic Packaging Materials Committee (FDCPMC) of the Society of the Plastics Industry (SPI) (PSWG, 1997) (attached as Appendix L) are summarized in Table 6-29.

Table 6-29. Weighted Average Residual Ethylbenzene Concentrations for All Applications of Polystyrene Packaging and Disposables ^a

Polymer/Applications	Residual Ethylbenzene (ppm)
Packaging	
GPPS	18
HIPS	29
PS foam	66
Disposables	
GPPS	42
HIPS	108
PS Foam	37
EPS Foam	37

^a Source: Based on “a survey of the industry” (PSWG, 1997; Appendix L).

6.4.3.1 Measured Migration

Daily Use Conditions

Durst and Laperle (1990) studied the migration of ethylbenzene from polystyrene containers into deionized water samples stored for up to 90 days at temperatures ranging from 24 to 66°C. Migration of ethylbenzene increased with time and storage temperature, ranging from 16 µg/L at all temperatures on day 1 to 209 µg/L on day 8 at a temperature of 66°C. The current relevance of these results is unknown, and no data on ethylbenzene levels in contemporary bottled water were found.

Citing a 1991 study by Matiella and Hsieh, Tang *et al.* (2000) reported that eggs stored in polystyrene package material had ethylbenzene concentrations ranging from 4 to 28 µg/kg. Jickells *et al.* (1992; cited in Tang *et al.* [2000]) noted that the rate of migration of ethylbenzene from thermoset polyester dishes was dependent on the fat content of the food. However, Ehret-Henry *et al.* (1994) reported that ethylbenzene concentrations in a range of yogurt samples were ≤ 4 µg/kg regardless of fat content. Styrene concentrations in these samples were higher, but also showed no consistent variation with yogurt fat content.

Melski *et al.* (2003) evaluated five objects commonly used for food packaging, of which three were made of (or included) polystyrene: a container cover, a cup, and foil. Refrigerated food contact was simulated by placing a piece of the material in a sealed vial with food simulant solutions of 3% acetic acid (acidic foods), 15% ethanol (alcoholic foods), or rectified olive oil (fatty foods) for 10 days at 5°C. The calculated migration reference values for ethylbenzene were approximately 11 and 80 mg/dm² in ethanol and olive oil, respectively; no migration was observed in acetic acid, indicating lower migration potential into acidic foods under these storage conditions.

The U.K. Food Standards Agency studied migration of ethylbenzene and other volatiles into 30 take-away foods and 40 snack foods intended to encompass the range of products and packaging materials available (Bradley *et al.*, 2004). These foods were expected to have a

relatively high migration potential due to their elevated temperature and high contact area:mass of food ratio, and estimates of worst-case migration potential suggested that the specific migration limit (SML) of 600 µg/kg in food set by the European Union could be exceeded. However, ethylbenzene was detected in only one of the foods tested (not specified in the on-line report) at a level of only 1 µg/kg. These data demonstrate that migration modeling can significantly overestimate potential exposure. The migrant chemicals in snack foods, which did not include ethylbenzene, derived principally from printing inks applied to the outside of the packaging. The authors concluded, "...even where potential migrants were present at comparatively high levels in the packaging of the snack foods the levels migrating into the foodstuffs were reassuringly low" (Bradley *et al.*, 2004).

Migration During Microwave or Other Cooking

Plastic containers that can be used in microwave ovens are increasingly popular, and migration of residual VOCs from microwaved styrenic materials has been recognized as a potential pathway for ethylbenzene exposure.

Wittrig (2002) evaluated VOC emissions from four materials comprising a single-serving microwavable bowl: an inner bowl containing the food item, a plastic lid, a printed shrink-wrapped label sampled at the seam, and the label sampled without the seam. The composition of these materials was not indicated. One-inch square samples were placed in purge and trap tubes and heated to 60°C for ten minutes (note that the FDA requires a temperature of at least 100°C). The highest levels of ethylbenzene and other VOCs derived from the label sampled at the seam; the lid emitted trace amounts, and the bowl no significant levels. Like those of Bradley *et al.* (2004), these results indicate that label inks/solvents can also be a source of VOCs in microwaved foods.

Gramshaw and Vandenburg (1995) evaluated the migration of ethylbenzene from thermoset polyester⁷ into pork belly during cooking at 175°C for 1 to 1.5 hours in a conventional oven. The concentration of ethylbenzene in five different samples of thermoset polyester ranged from 6 to 37 mg/kg. The migration value was estimated to range from <0.6 to 2.5 mg/dm² following first use, and from 1.8 to 5.5 mg/dm² on second and third uses. Concentrations of ethylbenzene in the cooked pork ranged from <6 to 20 µg/kg on first use, and increased to 25 to 34 µg/kg on subsequent uses. Migration of styrene also appeared to increase with multiple uses. The authors speculated that these increases might be due to differing weight losses in the meats being cooked, and less rigorous sealing of the dish in the first compared to later trials (Gramshaw and Vandenburg, 1995), although breakdown of the polymer with release of monomers was not ruled out. Leaving the dish uncovered resulted in substantially lower migration rates and concentrations in the meat, presumably due to volatilization into the oven. Overall, approximately 0.1% of the amount of ethylbenzene in the cookware migrated to the pork over the cooking time. The fat content of the meat samples was not reported, but the authors remarked that fat content was high, and may therefore be a more aggressive extractant than typically consumed meats (Gramshaw and Vandenburg, 1995).

⁷ A "dual-ovenable" material made by cross-linking chains of an unsaturated polyester with a third monomer, usually styrene, and designed for repeated use in both microwave and conventional ovens. There is a high residual styrene content after curing in the mold, which is reduced by a period of heating (Gramshaw and Vandenburg 1995).

Nerín *et al.* (2002) heated microwave-safe plastic containers made of polycarbonate, “polypropylene random,” “polypropylene copolymer” and polypropylene 20% talcum powder, and SAN⁸ to 100° C and measured volatile compounds released. Ethylbenzene was found in all container types evaluated, and was released upon heating from all but SAN (the material of greatest interest in terms of the ethylbenzene chain of commerce). The amounts of ethylbenzene released as vapor ranged from 0.15 to 0.36 µg per kg plastic. Assuming that migration to food is 100%, and accounting for the weight:surface ratio of each container, the projected concentrations of ethylbenzene in heated foods were 0.02 – 0.03 µg/kg, four orders of magnitude below the SML of 600 µg/kg.

Nerín and Acosta (2002) also studied the behavior of solid food simulants, Tenax® and Porapak®, in contact with SAN and other plastics used in microwave ovens. Each of the simulants was poured over 8 cm² of plastic, and the sample placed in a glass Petri dish and heated in a conventional oven (to ensure temperature control) for 30 minutes at 120°C. Estimated solid food concentrations of ethylbenzene in the two simulants were 0.0042 µg/kg for Tenax®, and 0.145 µg/kg for Porapak®. As noted by the authors, both of these concentrations are well below the SML of 600 µg/kg.

Melski *et al.* (2003) examined the effect of microwaving for four to 30 minutes on global migration of volatiles from the five polypropylene and/or polystyrene objects described in the preceding section into food simulant solutions of 3% acetic acid (acidic foods), 15% ethanol (alcoholic foods), or rectified olive oil (fatty foods). Compared with migration of total VOCs after ten days of cold storage, microwaving increased migration from all materials, ranging from a maximum of less than 150% of reference migration from a white polypropylene container to a maximum of around 425% of reference migration from a yellow polypropylene cup. The maximum increased migration from a polystyrene cup was 225%. As the migrant compounds were not distinguished, the contribution of ethylbenzene to these results could not be determined.

6.4.3.2 Estimating Migration Using Kinetic Modeling

Default approaches of migration of food contact substances (FCS) like that described in FDA (2002 and 2004b) make simplistic (and conservative) assumptions about the degree of migration of FCSs from food-contact materials. Kinetic modeling provides a means to incorporate time into the exposure estimation, thereby improving accuracy. The results of kinetic modeling performed by the PSWG (PSWG, 1997) (Appendix L) are presented here. The PSWG analysis is based on Lickly *et al.*'s (1995) modification of the FDA protocol to develop a detailed estimate of styrene migration from styrenic food-contact materials. This modeling effort was based on the relatively substantial body of measured migration data available for styrene from polystyrene. PSWG's application of this approach to ethylbenzene is predicated on the assumption that differences in migration behavior between these two structurally similar compounds are trivial (PSWG, 1997).

A comparison of key physicochemical, geometric, topological, and electronic properties (Table 6-30) shows that ethylbenzene and styrene are very similar in molecular weight and

⁸ It is noted that the composition of the materials used in this Spanish study was not described in detail, and is of uncertain comparability to materials used in the U.S.

volume, and differ by less than a factor of two for most parameters. However, it is noted that several of the characteristics in which the two compounds differ may be highly influential in terms of migration potential; in particular, ethylbenzene is only half as water-soluble as styrene (although they have similar octanol/water partition coefficients), and has a 50% higher vapor pressure and a 17-fold higher dipole moment than styrene. While the net impact (if any) of these differences on ethylbenzene's migration behavior vs. that of styrene is unknown, for this assessment their migration behavior was assumed to be identical. This could result in an over- or under-estimation of exposure.

Table 6-30. Comparison of Key Molecular Descriptors for Ethylbenzene and Styrene

Descriptor	Units	Ethylbenzene	Styrene	Source
Molecular weight	g/mol	106.2	104.2	
Aqueous solubility	mg/L	1.7E+02	3.1E+02	SRC (2005)
Boiling point	°C	136	145	
Henry's law constant	atm-m ³ /mol	7.9E-03	2.8E-03	
Vapor pressure	mm Hg	9.6E+00	6.1E+00	
Diffusivity in air	cm ² /s	7.5E-02	7.1E-02	EPA (2002a)
Diffusivity in water	cm ² /s	7.8E-06	8.0E-06	
Log K _{ow}	unitless	3.2	3.0	SRC (2005)
		3.2E+00	3.2E+00	Yaffe <i>et al.</i> (2002)
Molecular volume	Å ³	122.2	115.0	Huibers and Katritzky (1998)
Bonding Information Content	unitless	5.8E+00	4.0E+00	
Partial Negative Surface Area	Å ²	-1.2E+01	-1.9E+01	
Polarizability	AU	6.2E+01	6.6E+01	Yaffe <i>et al.</i> (2002)
Total Hybrid Dipole Moment	Debye	3.3E-01	2.0E-02	
Ionization Potential	kcal	9.4E+00	9.1E+00	
Average Polarizability	au	6.2E+01	6.6E+01	
Valence molecular connectivity index:				
First-order	unitless	3.0E+00	2.6E+00	
Second-order		1.8E+00	1.6E+00	
Fourth-order		7.1E-01	5.9E-01	

A figure presented by Ehret-Henry *et al.* (1994) shows very similar migration of ethylbenzene and styrene from packaging material into varying amounts of yogurt (Figure 6-6).

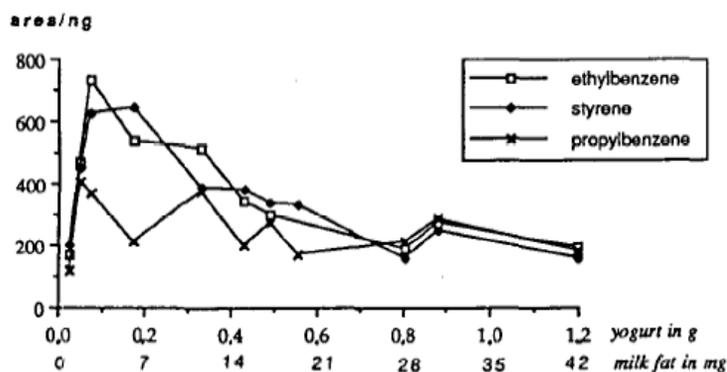


Figure 6-6. Effect of Yogurt Amounts on Hydrocarbon Desorption (from Ehret-Henry *et al.*, 1994)

6.4.3.1.1 Refined Consumption Factors

The FDA developed a consumption factor (CF) value for the category “polystyrene” of 0.1 based on information on the types of food consumed, the types of food contacting each packaging surface, the number of food packaging units in this food packaging category, the distribution of container sizes, and the ratio of the weight of food packaged to the weight of the package (FDA, 2002). Lickly *et al.* (1995) “subdivided” this general CF to develop refined CFs that more precisely represent the individual types of styrenic products, foods, and use conditions. However, these estimates were based on the FDA’s original CF of 0.08 rather than the rounded value of 0.1 adopted in 1995 (FDA, 1995). Therefore, PSWG increased Lickly *et al.*’s (1995) refined CFs by a factor of $0.1/0.08 = 1.25$ for use in ethylbenzene exposure assessment. These values are listed in Table 6-31.

6.4.3.1.2 Migration Factors

The degree of migration of a chemical from a polymer depends on its physicochemical properties and those of the packaging material, as well as the nature of the food and conditions of contact (temperature, duration). To account for their variable characteristics, FDA has grouped foods into four types depending on composition: aqueous, acidic, alcoholic and fatty (FDA, 2002). The “food-type distribution factors” (f_T) for each packaging material reflect the fraction of all food contacting each material that is of each of these types (FDA, 2002). However, in view of the varied exposure conditions identified for individual styrenic food-contact materials, as well as their varying styrene contents, Lickly *et al.* (1995) chose to calculate separate migration values for each individual condition based on Fick’s law of mass diffusion:

Table 6-31. Subdivided Consumption Factors Corrected for Rounding of the FDA's Generic Polystyrene Consumption Factor ^a

Polymer/Applications	Refined Consumption Factors
Packaging	
GPPS	
Produce baskets	0.0011
Pie containers	0.0011
Cookie trays	0.0105
HIPS	
Yogurt cups	0.0036
Cheese/cream containers	0.0036
Aseptic containers	0.0009
PS foam	
Egg cartons	0.0091
Stock food trays	0.0038
Total CF	0.034
Disposables	
GPPS (flatware and cutlery)	
Fatty: 75°F	0.0014
Fatty: 130°F	0.0001
Aqueous: 75°F	0.0043
Aqueous: 130°F	0.0005
HIPS ^b	
Fatty: 40°F	0.0001
Fatty: 75°F	0.0001
Fatty: 130°F	0.0003
Aqueous: 40°F	0.0108
Aqueous: 75°F	0.0188
Aqueous: 130°F	0.0016
Alcoholic	0.0015
PS Foam ^c	
Fatty: 75°F	0.003
Fatty: 130°F	0.0031
Aqueous: 75°F	0.006
Aqueous: 130°F	0.007
EPS Foam ^b	
Fatty: 75°F	0.0003
Fatty: 130°F	0.0006
Aqueous: 75°F	0.0018
Aqueous: 130°F	0.0051
Total CF	0.066
^a Source: PSWG (1997) (Appendix L) ^b Tumblers, cocktail glasses, vending and portion cups, dishes, plates, bowls, lids, closures, and flatware ^c Cups, containers, single-service plates, single-service hinged containers, food service trays and other foam sheet	

$$\frac{\partial c_t}{\partial t} = D_p \frac{\partial^2 c}{\partial x^2} \quad \{6-1\}$$

where:

- c_t = Concentration of migrant in the food-contact material (P) at time t at distance x from the origin of the x-axis
- D_p = Constant diffusion coefficient in the food-contact material

Equation {6-2} is applicable when equilibrium partitioning may have an effect on migration, such as with aqueous foods:

$$M_t = M_t^* \alpha K C_{p0} \quad \{6-2\}$$

where:

- M_t = migration at time t ($\mu\text{g}/\text{cm}^2$)
- M_t^* = ratio of the amount of substance migrating into aqueous food/simulants vs. the amount of substance that would migrate when equilibrium partitioning is reached
- C_{p0} = initial residual concentration of substance in polymer ($\mu\text{g}/\text{cm}^3$)
- α = volume of food simulant (mL/cm^2)
- K = partition coefficient of substance between polymer and food simulant

To solve this equation, Lickly *et al.* (1995) estimated M_t^* by calculating Z (Equation {6-3}), then extrapolating M_t^* from the curve presented in Figure 8 of Till *et al.* (1982).

$$Z = \frac{(D_p t)^{1/2}}{\alpha K} \quad \{6-3\}$$

where:

- Z = intermediate value (cm^3/mL)
- D_p = diffusion coefficient of substance in the polymer ($\text{cm}^2/\text{second}$)
- t = time of food contact (seconds)
- α = volume of food simulant (mL/cm^2)
- K = partition coefficient of substance between polymer and food simulant

Equation {6-4} is applicable when equilibrium partitioning has little effect on migration, such as with the migration of most organic substances into lipids or where there are very short exposure times to aqueous foods, as in the case of disposables:

$$M_{t1} = 2C_{p0} \left(D_p \frac{t}{\pi} \right)^{1/2} \quad \{6-4\}$$

where:

- M_{t1} = migration at time t ($\mu\text{g}/\text{cm}^2$)
- C_{p0} = initial residual concentration of substance in polymer ($\mu\text{g}/\text{cm}^3$)
- D_p = diffusion coefficient of substance in the polymer ($\text{cm}^2/\text{second}$)
- t = time of food contact (seconds)

Finally, for cases in which packaged food will be exposed to two different temperatures during the overall time in contact with packaging material (*e.g.*, a heat-sterilized foodstuff is subsequently kept refrigerated), migration at the second temperature can be calculated as:

$$M_{t2} = 2C_{p01} \left(\frac{1}{\pi} \right)^{1/2} \left[\left(D_{p1}t_1 + D_{p2}t_2 \right)^{1/2} - \left(D_{p1}t_1 \right)^{1/2} \right] \quad \{6-5\}$$

where the subscripts 1 and 2 indicate the diffusion coefficients and times for the first and second temperature regimes.

The total migration is then estimated as the sum of both phases:

$$M_t = M_{t1} + M_{t2} \quad \{6-6\}$$

It is evident from the above equations that the key parameters controlling migration of a substance from a plastic food-contact material into a food/simulant are: (1) the diffusion coefficient D_p (mobility) of the substance in the plastic; and, (2) the partition coefficient K (relative solubility at equilibrium) of the substance between the plastic and the food/simulant. Lickly *et al.* (1995) cited experimental data for styrene indicating a linear inverse relationship with temperature for GPPS, HIPS, and polystyrene foam according to the following regression relationships⁹:

- For GPPS (between 40 and 160°F),

$$\log D_p \text{ (GPPS)} = 2.724 - 4932 \left(\frac{1}{T} \right) \quad \{6-7\}$$

- For HIPS (between 70 and 150°F),

⁹ PSWG (1997) made a slight adjustment to the D_p values calculated by Lickly *et al.* (1995) for styrene using Equations {6-7} to {6-9} based on the relative molecular weights of the compounds (104.2 for styrene/106.2 for ethylbenzene = 0.98). This minor adjustment was considered unnecessary for this analysis.

$$\log D_p \text{ (HIPS)} = 1.9407 - 4623.7 \left(\frac{1}{T} \right) \quad \{6-8\}$$

- For polystyrene foam:

$$\log D_p \text{ (PF)} = 4.543 - 4407.6 \left(\frac{1}{T} \right) \quad \{6-9\}$$

where:

T = absolute temperature (°K)

A linear relationship also exists between the log of the partition coefficient K and the inverse of the absolute temperature for aqueous but not fatty food simulants. Lickly *et al.* (1995) presented the following equation for both GPPS and HIPS:

$$\log K = 2.2725 - 1773.9 \left(\frac{1}{T} \right) \quad \{6-10\}$$

6.4.3.1.3 Conversion of Residual Ethylbenzene Concentration in Packaging and Disposables

The residual ethylbenzene concentrations, as reported in Table 6-29, are provided in units of ppm (or mg/kg), while the equations specified in 6.4.3.1.2 require the initial residual concentration to be in units of $\mu\text{g}/\text{cm}^3$. This conversion was accomplished by multiplying the residual concentration in ppm by the average density in units of gm/cm^3 of the article being evaluated. For the GPPS and HIPS articles, a density of $1 \text{ gm}/\text{cm}^3$ was used with average densities of 0.2, 0.08, and $0.06 \text{ gm}/\text{cm}^3$ used for PS foam stock food trays, PS foam disposables, and EPS foam disposables, respectively.¹⁰

6.4.3.1.4 Adjustment for Mineral Oil Content

Lickly *et al.* (1995) modified the calculated styrene migration levels based on the results of experiments indicating that mineral oil used as a plasticizer in GPPS (4.5% mineral oil) and HIPS (3.5% mineral oil) increased migration from these materials into cooking oil at 40°C by factors of 3.16 and 5, respectively. However, a lower estimate of mineral oil content was provided in a 1996 report by the SPI's FDCPMC entitled "Report on Potential Exposure to Mineral Oil from Food-Contact Use of Polystyrene Resins" (cited in PSWG [1997]). This report indicated that:

- The weighted average mineral oil content of HIPS used for yogurt cups, cream/cheese containers, and aseptic containers was 2.5%, 2.8%, and 2.9%, respectively.

¹⁰ These average densities were not reported in PSWG (1997) but were back calculated from the known residual concentrations reported in Table 3 of PSWG (1997) and the migration values reported in Table 5 of PSWG (1997).

- The weighted average mineral oil content of GPPS and HIPS disposables was 1.3% and 2.5%, respectively.

On this basis, PSWG (1997) calculated proportionate decreases in the migration multipliers. The same adjustment was made here (although with slightly different results):

- For HIPS yogurt cups, the migration multiplier due to mineral oil content was reduced to 3.6-fold (5-fold increase/3.5% mineral oil content x 2.5% mineral oil content = 3.6);
- For HIPS cheese and cream containers, the migration multiplier due to mineral oil content was reduced to 4.0-fold (5-fold increase/3.5% mineral oil content x 2.8% mineral oil content = 4.0);
- For HIPS aseptic containers, the migration multiplier due to mineral oil content was reduced to 4.1-fold (5-fold increase/3.5% mineral oil content x 2.9% mineral oil content = 4.1);
- For GPPS disposables, the migration multiplier due to mineral oil content was reduced to one (3.16-fold increase/4.5% mineral oil content x 1.3% mineral oil content = 0.9¹¹); and,
- For HIPS disposables, the migration multiplier due to mineral oil content was reduced to 3.6-fold (5-fold increase/3.5% mineral oil content x 2.5% mineral oil content = 3.6).

The applicability of these nine-year old data to today's materials is unknown, and is noted as an uncertainty in this analysis.

6.4.3.1.5 Calculation of Migration of Ethylbenzene from Styrenic Food-contact materials into Food

The potential migration of ethylbenzene from styrenic materials into foodstuffs was calculated using the above equations and the time and temperature conditions for product use specified by Lickly *et al.* (1995) and also used by PSWG (1997). Results are presented in Table 6-32 and Table 6-33.

These results differ slightly from results presented in PSWG (1997) for cheese/cream containers, cookie trays, and aseptic containers, presumably due to differences in the M_t^* value obtained from Figure 8 of Till *et al.* (1982). The values in Table 6-33 for flatware and cutlery were lower than those presented in PSWG (1997) due to the different multipliers (1.0 in this report compared to 1.6 in PSWG [1997]) for mineral oil adjustment (see Section 6.4.3.1.4).

The total concentration of ethylbenzene estimated to be present in the diet due to migration from styrenic food-contact materials was 0.45 µg/kg (sum of subtotals in Table 6-32 and Table 6-33).

¹¹ Multiplier set at 1.0 since there is no reason to expect a lower migration for a slight amount of mineral oil in the polymer.

6.4.4 Summary

Ethylbenzene is seldom detected in the U.S. food supply, and only at low concentrations (fractions of a part per million). The literature reviewed for this study supports the following generalizations:

- Foods are potentially subject to accumulation of low levels of ethylbenzene by partitioning from ambient atmospheric sources as well as by migration from styrenic food-contact materials; and
- Moderate heating does not result in significant migration of ethylbenzene into packaged foods, but microwaving or other intense heating of food in styrenic (and other plastic) containers can increase the possibility of migration.

It is generally not possible to distinguish the unique contributions from these sources in the available data; therefore, it is assumed that the available measured food levels discussed in Section 6.4.1 reflect ethylbenzene inputs from all sources. The TDS data provided in Table 6-26 are used in Section 6.7.2.2 to estimate central tendency and upper-bound total daily dietary intakes of ethylbenzene. In order to evaluate the contribution from food-contact materials, the total dietary intake was compared to the intake estimated using the food concentration term derived from Lickly *et al.*'s (1995) kinetic migration model (0.45 µg/kg).

Table 6-32. Model for Estimating Daily Dietary Intake of Ethylbenzene from Food Packaging Materials

Material Type	Contact Time (days)	Temp (K)	Diffusion (D _p)	Eqn	Partition (K)	Eqn	Z ^a	Eqn	M _i * ^b	Migration (M _i) ^c	Eqn	CF ^d	Dietary Concentration (µg/kg)
GPSS													
Produce baskets	7	278	9.06E-16	{6-7}	7.63E-05	{6-10}	0.198	{6-3}	0.200	0.274	{6-2}	0.0011	0.0003
Pie containers	30	278	9.06E-16	{6-7}	7.63E-05	{6-10}	0.410	{6-3}	0.350	0.480	{6-2}	0.0011	0.0005
Cookie trays	60	297	1.32E-14	{6-7}	2.00E-04	{6-10}	0.844	{6-3}	0.540	1.941	{6-2}	0.0105	0.0204
HIPS													
Yogurt cups	30 min	339	1.95E-12	{6-8}						4.501	{6-4}	0.0036	
	60	278	1.92E-15	{6-8}						4.325	{6-5}	0.0036	
										8.826		0.0036	0.0318
Cheese/cream containers	30	278	1.92E-15	{6-8}	7.63E-05	{6-10}	0.598	{6-3}	0.440	3.891	{6-2}	0.0036	0.0140
Aseptic containers	60	297	2.37E-14	{6-8}	2.00E-04	{6-10}	1.132	{6-3}	0.590	14.011	{6-2}	0.0009	0.0126
PS foam													
Egg cartons	30	278								8.000	none	0.0038	0.0304
Stock food trays ^e	10	278	4.63E-12	{6-9}						19.210	{6-4}	0.0091	0.1748
Subtotal from food packaging												0.285	

Notes:

^a alpha = 1.55 mL/cm² (Using the FDA standard approximate volume to surface ratio of 10 mL/in²).

^b Value taken from Figure 8, Till *et al.* (1982)

^c Migration adjusted for mineral oil content as follows: 3.6 for yogurt cups; 4.0 for cheese and cream containers; 4.1 for aseptic containers.

^d Data from Tables 1 and 2, PSWG (1997)

^e Initial residual ethylbenzene concentration reported in Table 6-29 was adjusted by multiplying by the density of 0.2 gm/cm³.

Table 6-33. Model for Estimating Daily Dietary Intake of Ethylbenzene from Disposable Food-Contact Materials

Material Type	Contact Time (hours)	Temp (K)	Diffusion (D _p)	Eqn	Migration (M _t) ^a	Eqn	CF ^b	Dietary Concentration (µg/kg)
GPPS								
Flatware and cutlery	1	297	1.32E-14	{6-7}	0.211	{6-4}	0.0014	0.0003
	1	328	4.66E-13	{6-7}	1.253	{6-4}	0.0001	0.0001
	1	297	1.32E-14	{6-7}	0.211	{6-4}	0.0043	0.0009
	1	328	4.66E-13	{6-7}	1.253	{6-4}	0.0005	0.0006
HIPS								
Dishes, plates, glasses, etc.	1	278	1.92E-15	{6-8}	0.745	{6-4}	0.0001	0.0001
	1	297	2.37E-14	{6-8}	2.614	{6-4}	0.0001	0.0003
	1	328	6.71E-13	{6-8}	13.909	{6-4}	0.0003	0.0042
	1	278	1.92E-15	{6-8}	0.745	{6-4}	0.0108	0.0080
	1	297	2.37E-14	{6-8}	2.614	{6-4}	0.0188	0.0491
	1	328	6.71E-13	{6-8}	13.909	{6-4}	0.0016	0.0223
1	297	2.37E-14	{6-8}	2.614	{6-4}	0.0015	0.0039	
PS Foam^c								
Cups and containers	1	297	5.06E-11	{6-9}	0.920	{6-4}	0.003	0.0028
	1	328	1.23E-09	{6-9}	4.527	{6-4}	0.0031	0.0140
	1	297	5.06E-11	{6-9}	0.920	{6-4}	0.006	0.0055
	1	328	1.23E-09	{6-9}	4.527	{6-4}	0.007	0.0317
EPS Foam^c								
Cups and containers	1	297	5.06E-11	{6-9}	0.690	{6-4}	0.0003	0.0002
	1	328	1.23E-09	{6-9}	3.396	{6-4}	0.0006	0.0020
	1	297	5.06E-11	{6-9}	0.690	{6-4}	0.0018	0.0012
	1	328	1.23E-09	{6-9}	3.396	{6-4}	0.0051	0.0173
Subtotal from disposables								0.165
Notes:								
^a Migration adjusted for mineral oil content as follows: 3.6 for HIPS disposables								
^b Data from Tables 1 and 2, PSWG (1997)								
^c Initial residual ethylbenzene concentrations reported in Table 6-29 were adjusted using a density of 0.08 gm/cm ³ and 0.06 gm/cm ³ for PS Foam and EPS Foam, respectively.								

6.5 Migration of Ethylbenzene from Plastic

Commercial styrene-containing polymers, such as polystyrene and acrylonitrile-butadiene-styrene (ABS), contain residual amounts of ethylbenzene from the production process. These polymers are used not only in food packaging materials, but also in children’s toys, such as balls, playmats, and gym sets, as well as in common household objects. Toys designed for mouthing by young children, such as teething rings, are typically made from polyvinyl chloride (PVC) (Steiner *et al.*, 1998) rather than styrene-containing polymers. However, in the absence of product-specific information and for purposes of this Tier 1 exposure assessment, several conservative assumptions were made:

- All non-pacifier objects mouthed by young children are made of styrene-containing polymers; and,
- The migration rate of ethylbenzene from toys does not decrease over the age interval for which exposure is estimated (*i.e.*, mass balance is ignored after the toy is purchased).

6.5.1 Estimation of Daily Migration Rate

In order to estimate potential transfer of ethylbenzene from toys made of styrene-containing polymers, a simple model based on that used to estimate transfer of styrene from polymeric packaging materials to food was used (Lickly *et al.*, 1995). Using this model, it was assumed that ethylbenzene migrates from polystyrene materials into the saliva in the mouth of a child in a manner consistent with Fick's diffusion theory (Till *et al.*, 1982). When equilibrium partitioning has little effect, such as with the migration of ethylbenzene into oil or aqueous environments, Fick's diffusion theory equation reduces to:

$$M_t = 2 \times C_{p0} \times \left(\frac{D_p \times t}{\pi} \right)^{1/2} \quad \{6-11\}$$

where:

M_t	=	cumulative mass migration of ethylbenzene over time ($\mu\text{g}/\text{cm}^2$)
C_{p0}	=	initial residual ethylbenzene level in polymer ($\mu\text{g}/\text{cm}^3$)
D_p	=	diffusion coefficient of ethylbenzene in the polymer (cm^2/sec)
t	=	time over which mass release is calculated (seconds)

Therefore, if the initial residual concentration of ethylbenzene in the polystyrene material, diffusion coefficient, and age of the object are known, then the amount of ethylbenzene that may potentially migrate from the object into the saliva of the child's mouth at a given time can be estimated.

Residual concentrations of ethylbenzene have been measured for all applications of polystyrene packaging and disposables (PSWG, 1997) (see Section 6.4.3.2). These concentrations ranged from 18 ppm in GPPS to 108 ppm in disposable HIPS materials (Table 6-29). In the absence of product-specific information, it was assumed that mouthable toys are more likely to be made of non-disposable HIPS, with a weighted average ethylbenzene concentration of 29 ppm. With an assumed density of $1 \text{ gm}/\text{cm}^3$, this results in an initial residual ethylbenzene concentration of $27 \mu\text{g}/\text{cm}^3$.

No information was available in the literature on the diffusion coefficient for ethylbenzene from polystyrene materials. However, as the general diffusion of ethylbenzene is expected to be similar to that of styrene, based on their structural similarities (see Section 6.4.3.2), the following discussion assumes equivalence. A linear relationship exists between the log of the apparent diffusion coefficient of ethylbenzene and the inverse of the absolute temperature of the styrenic material. Therefore, for a given temperature, the diffusion coefficient for styrene from the material can be estimated using Equation {6-8}. At body temperature, the estimated diffusion coefficient is $1.08 \times 10^{-13} \text{ cm}^2/\text{sec}$.

Determining the migration of ethylbenzene from a toy is dependent on the age of the toy, because the migration of the compound in the toy will decrease as the toy ages. It is conservatively assumed that toys in high demand may only be two months old at the time of purchase (4 weeks between manufacture and shelf placement and 4 weeks between shelf placement and purchase). Based on an assumed average "age" of two months at purchase, a

daily migration of 0.0002 $\mu\text{g}/(\text{cm}^2\text{-day})$ on the day of purchase (*i.e.*, the daily migration for the first day of the second month) was estimated using the following equation:

$$\begin{aligned} \text{DMR} &= M_t(2 \text{ months} + 1 \text{ day}) - M_t(2 \text{ months}) \\ &= \left[2 \cdot 29 \cdot \left(1.08 \times 10^{-13} \cdot \frac{5,342,400}{\pi} \right)^{1/2} \right] - \left[2 \cdot 29 \cdot \left(1.1 \times 10^{-13} \cdot \frac{5,256,000}{\pi} \right)^{1/2} \right] \quad \{6-12\} \\ &= 0.0249 - 0.0247 = 0.0002 \frac{\mu\text{g}}{\text{cm}^2\text{-day}} \end{aligned}$$

where:

DMR	=	Daily migration rate of ethylbenzene from the toy or object when the object is two months old ($\mu\text{g}/\text{cm}^2\text{-day}$)
M_t	=	cumulative mass migration of ethylbenzene at the designated time ($\mu\text{g}/\text{cm}^2$); estimated using Equation {6-11}
C_{p0}	=	29 $\mu\text{g}/\text{cm}^3$ for non-disposable HIPS (Table 6-29)
D_p	=	$1.08 \times 10^{-13} \text{ cm}^2/\text{sec}$ (calculated per Equation {6-8} at body temperature)
$t_{2 \text{ mos.} + 1 \text{ day}}$	=	$(365 \text{ days/year} \cdot 2/12 \text{ year} \cdot 24 \text{ hours/day} \cdot 3,600 \text{ sec/hour}) + (24 \text{ hours/day} \cdot 3,600 \text{ sec/hour}) = 5,342,400 \text{ seconds}$
$t_{2 \text{ mos.}}$	=	$(365 \text{ days/year} \cdot 2/12 \text{ year} \cdot 24 \text{ hours/day} \cdot 3,600 \text{ sec/hour}) = 5,256,000 \text{ seconds}$

The DMR estimated using Equation {6-12} is used in Section 6.7.3 to estimate a child's intake due to mouthing of objects.

6.6 Exposure Pathway Model

6.6.1 Overview of Approach

Because ethylbenzene is both naturally occurring and industrially important, it is ubiquitous in the general environment. As a result, virtually everyone in the U.S. may be exposed to the compound from a variety of sources via multiple pathways. The dominant exposure route for both workers and the general public is inhalation due to ethylbenzene's propensity to partition to the atmosphere, regardless of emission mode (see Section 5.2.2). As shown in Table 5-7 (see also Section 5.3), the majority of ethylbenzene emissions originate from sources outside the ethylbenzene/styrene chain of commerce, including biomass burning, petroleum-related industries, production and use of mixed xylene solvents, tobacco smoke, etc.

The objectives of the exposure assessment are: (1) to document the sources and means by which children and prospective parents could be exposed to ethylbenzene; (2) to develop conservative but balanced and broadly applicable estimates of the potential total ethylbenzene exposure to children and prospective parents; and, (3) to determine the proportional contribution of different exposure pathways to the total exposure. An additional

objective of this assessment was to distinguish, on a semi-quantitative basis, that proportion of each exposure pathway that is directly attributable to the ethylbenzene/styrene chain of commerce. Evaluation of exposures to ethylbenzene that occur as part of an exposure to mixed xylenes was not considered since the exposures are not related to the ethylbenzene chain of commerce. The uncertainty related to their exclusion is presented in Section 9.6.1.

This section provides the conceptual basis for the approach taken in this Tier 1 exposure assessment, as well as the exposure assumptions and parameter values used to characterize potential exposures to children and prospective parents.

6.6.2 Conceptual Exposure Model

The strategy used to evaluate exposures to ethylbenzene involved elements of both chain of commerce and receptor-centered approaches, as summarized in Table 6-34.

Inhalation of ethylbenzene vapors is the major pathway for most populations, so attention was focused on indoor and outdoor air, including motor vehicles. As ethylbenzene may be present in food, dietary intakes were also considered. It is recognized that spills and releases can result in local contamination of soil, sediment, surface water, and groundwater. However, such isolated conditions do not reflect those experienced on a long-term basis by the general public. Thus, potential exposures via contact with surface water, and groundwater were not quantified in this assessment because, as noted in a number of national databases, ethylbenzene was infrequently detected in these media and, when detected, was present at very low concentrations. Potential exposures via contact with soil and sediment were not quantified because the data available for these media are consistent with the reasonable expectation based on knowledge of the environmental fate of ethylbenzene that they are insignificant repositories and hence unimportant as potential exposure media (see Sections 5.2 and 6.2).

Table 6-34. Strategies for Evaluating Exposure to Ethylbenzene

Exposure Assessment Approach	Exposure Source
Chain of commerce	Occupational settings
	Industrial releases
	Polystyrene plastics in consumer products
	Diet (from packaging materials)
Receptor-centered	Outdoor air
	Indoor air (home, work, and school)
	In-vehicle and other automotive-related exposures
	Drinking water
	Diet (fresh food, breastfeeding)

6.6.3 Exposure Scenarios

As indicated in Table 6-34, individuals exposed to the compound by way of the ethylbenzene/styrene chain of commerce include workers involved in ethylbenzene production and styrene/polystyrene manufacture, and the general public exposed to ambient environmental releases from these industries as well as migration or off-gassing from

styrenic products. The following populations could be examined for potential exposure to ethylbenzene in the ethylbenzene/styrene chain of commerce:

- Prospective parents engaged in the manufacture of ethylbenzene, styrene, and styrenic products;
- Breast-fed infants of occupationally exposed mothers;
- Young children mouthing toys composed of styrenic materials;
- Prospective parents and children living, working, and going to school in buildings made with styrenic structural components and/or containing styrenic appliances, electronic devices, etc.¹²; and
- Prospective parents and children consuming foods contacting styrenic materials.

However, with the exception of limited workplace air concentrations, data are not available to distinguish the original source(s) of ethylbenzene levels in potential exposure media, such as air and food. Environmental monitoring data necessarily combine inputs from general and industrial sources with those from ethylbenzene/styrene chain of commerce sources associated with production and use of the neat compound and consumer uses of the wide array of styrenic materials.

Thus, the approach taken in this Tier 1 exposure assessment was to use the most recent available national air and food monitoring data to develop central tendency and upper-bound estimates of defined populations' total exposures via inhalation and ingestion, and then use emission contribution estimates and the models predicting migration from food-contact materials to estimate the proportion of total exposure that may be attributable to the ethylbenzene/styrene chain of commerce.

The exposure scenarios developed for these purposes are described in the following sections.

6.6.3.1 Exposure Settings

Given the higher ambient concentrations of ethylbenzene in urban air, both urban and rural/suburban settings were considered. Given the contribution of ETS to ethylbenzene levels in indoor air, both smoking and non-smoking homes were considered. Thus, four broad exposure regimes, or settings, were identified:

- Urban, Smoking;
- Urban, Non-smoking;
- Rural/suburban, Smoking; and
- Rural/suburban, Non-smoking.

¹² Contribution of these sources could not be quantitatively evaluated due to lack of data. Therefore, they were evaluated semi-quantitatively through measured indoor and outdoor air concentration ratios and an estimated contribution to outdoor air by ethylbenzene/styrene chain of commerce facilities.

6.6.3.2 Receptor Populations

The following receptor populations were identified:

- Children aged 0 to 19 years, divided into six age classes (< 1 year, 1 – 2 years; 3 – 5 years; 6 – 8 years; 9 – 14 years; 15 – 19 years) based upon FDA ingestion rates and
- Prospective parents of reproductive age (*i.e.*, assumed to be aged 19 to 45 years).

6.6.3.3 Receptor Microenvironments

Each of these populations was considered to live and work within one of these settings. Individuals were considered to be either outdoors or indoors in one of five primary locations:

- Home;
- School;
- Work (production facility or other);
- Motor vehicle; and
- Outdoors.

6.6.3.4 Exposure Pathways

Exposure pathways evaluated for these groups are summarized in Figure 6-7 and Table 6-35. The primary exposure route for all scenarios was inhalation of ethylbenzene vapors, which can occur in all microenvironments. Because of its volatility, ethylbenzene is not expected to be introduced into workers' homes via contaminated clothing, so exposures for production workers and their families were limited to direct inhalation while at work and partitioning to mother's milk, respectively. All individuals were assumed to be exposed via the diet. The only exposure pathways unique to young children were ingestion of mother's milk and mouthing of styrenic toys.

6.6.4 Exposure Assumptions and Values

To provide a conservative but balanced and broadly applicable representation of potential ethylbenzene exposures in the U.S. public, this Tier 1 exposure assessment combined generally central tendency exposure parameter values (*e.g.*, inhalation rates, food intake) with central tendency and upper-bound ethylbenzene concentrations in exposure media (air, food). As discussed previously, distinction was made among significant exposure settings (urban vs. rural/suburban, smoking vs. non-smoking) and microenvironments (homes, workplaces, schools).

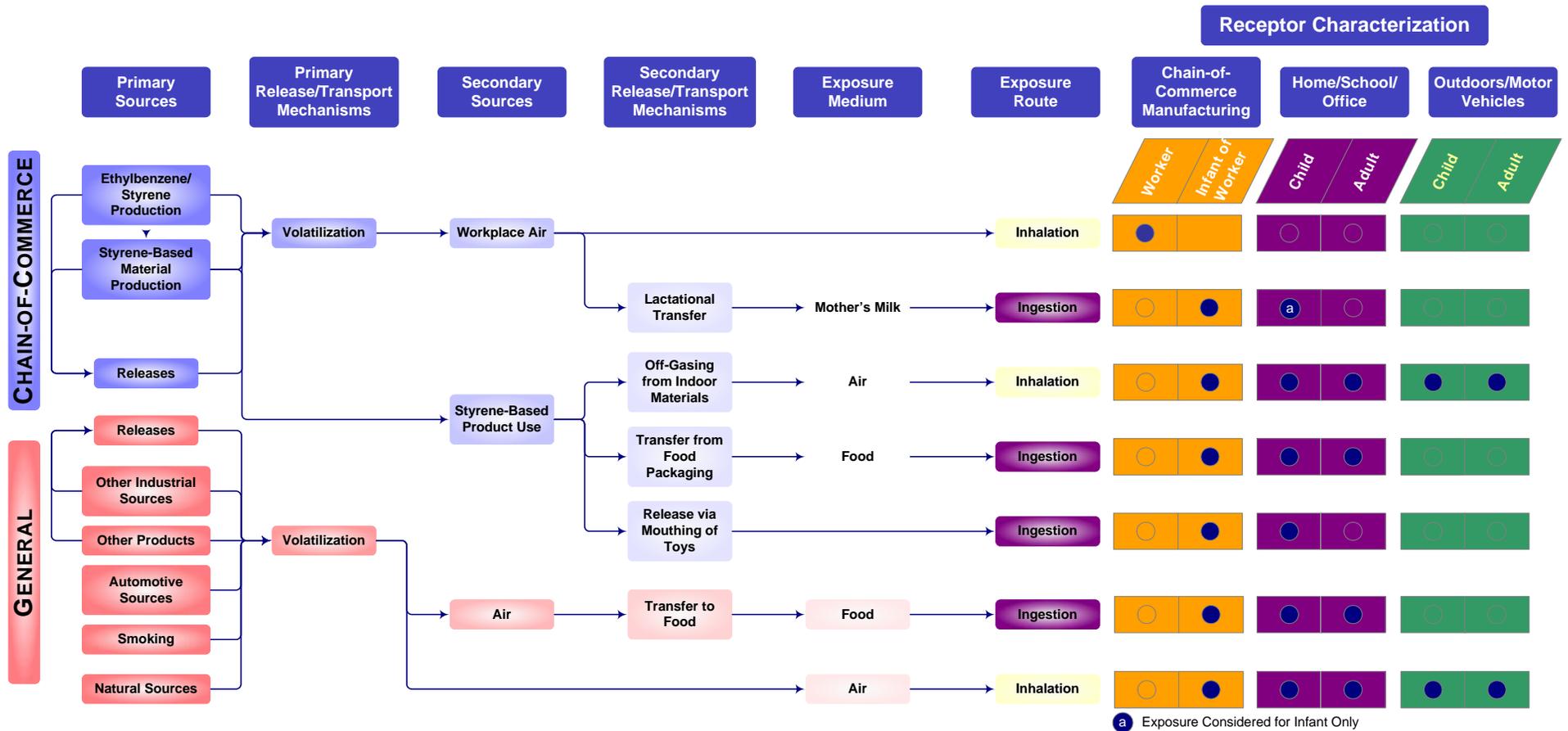


Figure 6-7. Conceptual Exposure Pathway Model for Exposure of Children and Prospective Parents to Ethylbenzene from Chain of Commerce and General Sources

Table 6-35. Exposure Pathways Examined for Representative Populations

Population	Chain of Commerce							Other Sources			
	Inhalation				Ingestion			Inhalation			Ingestion
	Workplace	Indoor	Ambient	Motor Vehicles	Mother's Milk	Food	Migration from Toys	Indoor	Ambient	Motor Vehicles	Food
<1 (breastfed)		•	•	•	•	•	•	•	•	•	•
< 1 (not breastfed)		•	•	•		•	•	•	•	•	•
1-2		•	•	•		•	•	•	•	•	•
3-5		•	•	•		•	•	•	•	•	•
6-8		•	•	•		•		•	•	•	•
9-14		•	•	•		•		•	•	•	•
15-19		•	•	•		•		•	•	•	•
At-home adult (20-45)		•	•	•		•		•	•	•	•
Production Worker	•	•	•	•		•		•	•	•	•
Office Worker		•	•	•		•		•	•	•	•

6.6.4.1 Receptor-Specific Exposure Parameter Values

The exposure assumptions and values used to calculate ethylbenzene exposures for each population in each microenvironment are derived from applicable EPA and FDA guidance, as summarized in Table 6-36, Table 6-37, and Table 6-38. Estimated hours per day spent in different microenvironments were slightly adjusted to: (1) ensure that all times summed to 24 hours; and, (2) accommodate age groupings used in this analysis. In addition, daily hours spent at work and school were adjusted for weekends and vacations (during which all individuals were assumed to be at home). Similarly, microenvironment-specific inhalation volumes were derived by adjusting age group-specific daily inhalation rates for time spent in the microenvironment.

Table 6-36. Age-Group Specific Body Weights and Inhalation Rates^a

Age Group (years)	Body Weight (kg)	Inhalation Rate (m ³ /day)
0-1	8.5	4.5
1-2	12.2	6.8
3-5	17.2	8.3
6-8	25.1	10
9-14	45.5	13.5
15-19	67.8	14.5
20-45	71.8	16

^a Source: EPA (1997a, 2002b)

Table 6-37. Age Group-Specific Body Weights and Total Food Consumption Rates

Age Group (years)	Body Weight (kg) ^a	Total Food Consumption (g/day) ^b
<1	9.4	1,158
2	12.9	1,315
6	21.7	1,483
10	35.6	1,672
14-16	63.2	1,698 (female) 2,468 (male) 2,083 (average)
25-30	71.5	1,923 (female) 2,711 (male) 2,317 (average)
40-45	74	2,034 (female) 2,757 (male) 2,396 (average)

^a Source: EPA (1997a, 2002b)

^b Source: FDA (2004b)

Table 6-38. Summary of Age Group- and Microenvironment-Specific Exposure Times and Inhalation Rates

Age Group	Home		Work		School		Motor Vehicle		Outdoors	
	Exposure Time (hours/day)	Inhalation (m ³ /day)	Exposure Time (hours/day) ^a	Inhalation (m ³ /day)	Exposure Time (hours/day) ^b	Inhalation (m ³ /day)	Exposure Time (hours/day)	Inhalation (m ³ /day)	Exposure Time (hours/day)	Inhalation (m ³ /day)
<i>Child^c</i>										
<1 year	22.00	4.13	NA	NA	NA	NA	1.0	0.19	1.0	0.19
1-2	20.40	5.78	NA	NA	1.10	0.31	1.0	0.28	1.5	0.43
3-5	16.76	5.80	NA	NA	2.74	0.95	1.5	0.52	3.0	1.04
6-8	15.66	6.53	NA	NA	3.84	1.60	1.5	0.63	3.0	1.25
9-14	15.66	8.81	NA	NA	3.84	2.16	1.5	0.84	3.0	1.69
15-19	16.66	10.07	NA	NA	3.84	2.32	1.5	0.91	2.0	1.21
<i>Adult^d</i>										
At-Home	21.00	14.00	NA	NA	NA	NA	1.5	1.00	1.5	1.00
Production Worker	15.52	10.35	5.5	3.65	NA	NA	1.5	1.00	1.5	1.00
Office Worker	15.52	10.35	5.5	3.65	NA	NA	1.5	1.00	1.5	1.00

^a Daily hours at work (assumed to be 8 hours/day on work days) adjusted by 250/365 for weekends and holidays (assumed to be spent at home)

^b Daily hours at school (age-specific from EPA [2002b]) adjusted by 200/365 for weekends and holidays (assumed to be spent at home)

^c Time and inhalation data from EPA (2002b)

^d Time and inhalation data from EPA (1997a&b)

NA – not applicable

6.6.4.2 Exposure Concentrations

Potential exposure to ethylbenzene can be estimated in several ways:

- Measured at the point of contact while it is taking place (*e.g.*, monitoring data);
- Estimated via predictive modeling using standard receptor scenarios; or,
- Reconstructed using biomarkers of exposure to estimate internal dose.

All three approaches were used to estimate central tendency and upper-bound exposures for the identified populations. Inhalation and total dietary exposure estimates were based on monitoring data, and migration modeling was used to estimate the proportion of dietary ethylbenzene contributed by styrenic food-contact materials and mouthing of toys.

Finally, exposure biomarker data (blood concentrations) were used to estimate lactational transfer.

6.6.4.2.1 Air

Ethylbenzene concentrations vary in different microenvironments. Central tendency and upper-bound exposure concentrations in air identified in Section 6.3.4 are summarized in Table 6-39.

6.6.4.2.2 Food

Diet

Mean and maximum concentrations of ethylbenzene in TDS food items presented in Table 6-26 were used as central tendency and upper-bound exposure concentrations in food.

Human Milk

As discussed in Section 6.1.3, estimated central tendency and upper-bound concentrations of ethylbenzene in human milk were estimated using a PBPK model. Levels used in the exposure assessment are summarized in Table 6-40.

6.6.4.2.3 Migration from Styrenic Materials

Food Contact Materials

Residual concentrations of ethylbenzene contained within polystyrene containers used as packaging for numerous foods and food products may migrate from the packaging to the food. This migration potential was estimated at 0.45 $\mu\text{g}/\text{kg}$ using a kinetic model (see Section 6.4.3.2).

Table 6-39. Summary of Setting- and Microenvironment-Specific Ethylbenzene Concentrations in Air ($\mu\text{g}/\text{m}^3$)

Setting/ Micro- environment	Urban, Smoking		Urban, Non-Smoking		Rural/Suburban, Smoking		Rural/Suburban, Non-Smoking	
	Central Tendency	Upper-Bound	Central Tendency	Upper-Bound	Central Tendency	Upper-Bound	Central Tendency	Upper-Bound
Home	5.1E+00	1.1E+01	3.4E+00	7.5E+00	2.7E+00	7.2E+00	1.8E+00	4.8E+00
School ^a	1.6E+00	3.6E+00	1.6E+00	3.6E+00	1.2E+00	3.2E+00	1.2E+00	3.2E+00
Outdoor ^a	1.1E+00	2.4E+00	1.1E+00	2.4E+00	5.9E-01	1.5E+00	5.9E-01	1.5E+00
Vehicle	6.9E+00	1.5E+01	4.6E+00	1.0E+01	3.7E+00	9.7E+00	2.5E+00	6.5E+00
Office	2.4E+00	5.4E+00	1.6E+00	3.6E+00	1.8E+00	4.8E+00	1.2E+00	3.2E+00
Industry ^a	4.3E+02	4.3E+03	4.3E+02	4.3E+03	4.3E+02	4.3E+03	4.3E+02	4.3E+03

^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for both columns.

Table 6-40. Estimated Concentrations of Ethylbenzene in Human Milk (µg/L)

Receptor Population	Predicted Milk Concentration (µg/L) ^a
General Population	
Central Tendency	0.11
Upper Bound	0.25
Production Worker	
Central Tendency	2.2
Upper Bound	21.0
^s See Section 6.1.3.	

Mouthing of Toys

As discussed in Section 6.5 ethylbenzene can migrate from polymers used in children’s toys, such as balls, playmats, and gym sets, as well as in common household objects. These toys may then be mouthed by young children. The daily migration rate of ethylbenzene from a toy or object that is 2 months old was estimated at 0.0002 µg/ cm²-day (see Section 6.5.1).

6.7 Development of Tier 1 Estimates of Ethylbenzene Exposure for Children and Prospective Parents

In this section, estimated exposure concentrations and parameter values developed in preceding sections were combined to estimate potential exposures to child and prospective parent receptor populations, as defined in the conceptual exposure model. In the current absence of carcinogenic toxicity criteria for this compound, intakes were expressed as average daily doses. Both total intakes and the potential ethylbenzene/styrene chain of commerce contributions to specific exposure pathways were evaluated.

6.7.1 Inhalation Exposure

Central tendency and upper-bound estimates of exposure to ethylbenzene via inhalation were calculated for all population groups in all microenvironments (at home, at work/school, outdoor, and riding in a motor vehicle) according to the following equation:

$$\text{Intake}_{\text{Inh}/r,\mu} \left[\frac{\text{mg}}{\text{kg-day}} \right] = \frac{[\text{EB}]_{\text{air}/S,\mu} \times \frac{\text{ET}_{r,\mu}}{24 \frac{\text{hours}}{\text{day}}} \times \text{DIR}_r \times 10^{-3} \text{ mg}/\mu\text{g}}{\text{BW}_r} \quad \{6-13\}$$

where,

Intake_{Inh} = average daily intake of ethylbenzene from inhalation exposure (mg/kg-day)

- [EB]_{air/S,μ} = concentration of ethylbenzene in air for each microenvironment in each exposure setting (μg/m³)
- ET_{r,μ} = population- and microenvironment-specific exposure time (hours/day)
- DIR_r = population-specific daily inhalation rate (m³/day)
- BW_r = receptor-specific body weight (kg)

Estimated daily ethylbenzene intakes via inhalation for child and adult groups in each setting and microenvironment are presented in the following sections. Daily intakes in all microenvironments were summed for each receptor group in each setting.

6.7.1.1 Total Inhalation Intake

Table 6-41 to Table 6-48 present inhalation intake results for children and adults in all microenvironments in Urban and Rural/Suburban settings, Smoking and Non-Smoking. As expected, Urban and Smoking exposures were higher than Rural/Suburban and Non-Smoking exposures, and children’s exposures were generally higher than adults’ due to their higher rate of inhalation per unit body weight. However, the Production Worker scenario had the highest total intake.

Table 6-41. Urban, Smoking: Ethylbenzene Inhalation by Children (mg/kg-day)

Microenvironment	<1 year	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years
Central Tendency						
Home	2.46E-03	2.40E-03	1.71E-03	1.32E-03	9.80E-04	7.50E-04
School ^a	NE	4.16E-05	9.01E-05	1.04E-04	7.75E-05	5.59E-05
Outdoor ^a	2.40E-05	3.80E-05	6.57E-05	5.43E-05	4.04E-05	1.94E-05
Vehicle	1.51E-04	1.59E-04	2.07E-04	1.71E-04	1.27E-04	9.18E-05
Total	2.64E-03	2.64E-03	2.07E-03	1.65E-03	1.22E-03	9.17E-04
Upper-Bound						
Home	5.48E-03	5.36E-03	3.81E-03	2.94E-03	2.19E-03	1.68E-03
School ^a	NE	9.28E-05	2.01E-04	2.32E-04	1.73E-04	1.25E-04
Outdoor ^a	5.36E-05	8.47E-05	1.47E-04	1.21E-04	9.01E-05	4.33E-05
Vehicle	3.38E-04	3.56E-04	4.62E-04	3.81E-04	2.84E-04	2.05E-04
Total	5.87E-03	5.89E-03	4.62E-03	3.67E-03	2.74E-03	2.05E-03
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for these microenvironments in Table 6-41 and Table 6-43. NE – Not estimated.						

Table 6-42. Urban, Smoking: Ethylbenzene Inhalation by Adults (mg/kg-day)

Microenvironment	At-Home Parent	Office Worker	Production Worker
<i>Central Tendency</i>			
Home	9.86E-04	7.32E-04	7.32E-04
Office	NE	1.25E-04	NE
Industry ^a	NE	NE	2.21E-02
Outdoor ^a	1.52E-05	1.52E-05	1.52E-05
Vehicle	9.56E-05	9.56E-05	9.56E-05
Total	1.10E-03	9.67E-04	2.29E-02
<i>Upper-Bound</i>			
Home	2.20E-03	1.63E-03	1.63E-03
Office	NE	2.78E-04	NE
Industry ^a	NE	NE	2.21E-01
Outdoor ^a	3.38E-05	3.38E-05	3.38E-05
Vehicle	2.13E-04	2.13E-04	2.13E-04
Total	2.45E-03	2.16E-03	2.23E-01
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for these microenvironments in Table 6-42 and Table 6-44. NE – Not estimated.			

Table 6-43. Urban, Non-Smoking: Ethylbenzene Inhalation by Children (mg/kg-day)

Microenvironment	<1 year	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years
<i>Central Tendency</i>						
Home	1.64E-03	1.60E-03	1.14E-03	8.80E-04	6.57E-04	5.02E-04
School ^a	NE	4.16E-05	9.01E-05	1.04E-04	7.75E-05	5.59E-05
Outdoor ^a	2.40E-05	3.80E-05	6.57E-05	5.43E-05	4.04E-05	1.94E-05
Vehicle	1.01E-04	1.06E-04	1.38E-04	1.14E-04	8.49E-05	6.12E-05
Total	1.76E-03	1.79E-03	1.43E-03	1.15E-03	8.60E-04	6.38E-04
<i>Upper-Bound</i>						
Home	3.66E-03	3.57E-03	2.54E-03	1.96E-03	1.46E-03	1.12E-03
School ^a	NE	9.28E-05	2.01E-04	2.32E-04	1.73E-04	1.25E-04
Outdoor ^a	5.36E-05	8.47E-05	1.47E-04	1.21E-04	9.01E-05	4.33E-05
Vehicle	2.25E-04	2.37E-04	3.08E-04	2.54E-04	1.89E-04	1.36E-04
Total	3.94E-03	3.99E-03	3.19E-03	2.57E-03	1.91E-03	1.42E-03
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for these microenvironments in Table 6-41 and Table 6-43. NE – Not estimated.						

Table 6-44. Urban, Non-Smoking: Ethylbenzene Inhalation by Adults (mg/kg-day)

Microenvironment	At-Home Parent	Office Worker	Production Worker
Central Tendency			
Home	6.57E-04	4.87E-04	4.87E-04
Office	NE	8.32E-05	NE
Industry ^a	NE	NE	2.21E-02
Outdoor ^a	1.52E-05	1.52E-05	1.52E-05
Vehicle	6.38E-05	6.38E-05	6.38E-05
Total	7.36E-04	6.49E-04	2.27E-02
Upper-Bound			
Home	1.47E-03	1.09E-03	1.09E-03
Office	NE	1.85E-04	NE
Industry ^a	NE	NE	2.21E-01
Outdoor ^a	3.38E-05	3.38E-05	3.38E-05
Vehicle	1.42E-04	1.42E-04	1.42E-04
Total	1.65E-03	1.45E-03	2.22E-01
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for these microenvironments in Table 6-42 and Table 6-44. NE – Not estimated.			

Table 6-45. Rural/Suburban, Smoking: Ethylbenzene Inhalation by Children (mg/kg-day)

Microenvironment	<1 year	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years
Central Tendency						
Home	1.33E-03	1.30E-03	9.24E-04	7.13E-04	5.31E-04	4.07E-04
School ^a	NE	3.15E-05	6.83E-05	7.89E-05	5.88E-05	4.23E-05
Outdoor ^a	1.30E-05	2.06E-05	3.56E-05	2.94E-05	2.19E-05	1.05E-05
Vehicle	8.20E-05	8.63E-05	1.12E-04	9.26E-05	6.89E-05	4.97E-05
Total	1.43E-03	1.44E-03	1.14E-03	9.14E-04	6.81E-04	5.10E-04
Upper-Bound						
Home	3.48E-03	3.39E-03	2.41E-03	1.86E-03	1.39E-03	1.07E-03
School ^a	NE	8.23E-05	1.78E-04	2.06E-04	1.53E-04	1.11E-04
Outdoor ^a	3.40E-05	5.36E-05	9.29E-05	7.67E-05	5.71E-05	2.74E-05
Vehicle	2.14E-04	2.25E-04	2.93E-04	2.42E-04	1.80E-04	1.30E-04
Total	3.73E-03	3.75E-03	2.98E-03	2.38E-03	1.78E-03	1.33E-03
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for both columns. NE – Not estimated.						

Table 6-46. Rural/Suburban, Smoking: Ethylbenzene Inhalation by Adults (mg/kg-day)

Microenvironment	At-Home Parent	Office Worker	Production Worker
<i>Central Tendency</i>			
Home	5.35E-04	3.96E-04	3.96E-04
Office	NE	9.46E-05	NE
Industry ^a	NE	NE	2.21E-02
Outdoor ^a	8.22E-06	8.22E-06	8.22E-06
Vehicle	5.18E-05	5.18E-05	5.18E-05
Total	5.95E-04	5.50E-04	2.26E-02
<i>Upper-Bound</i>			
Home	1.40E-03	1.03E-03	1.03E-03
Office	NE	2.47E-04	NE
Industry ^a	NE	NE	2.21E-01
Outdoor ^a	2.14E-05	2.14E-05	2.14E-05
Vehicle	1.35E-04	1.35E-04	1.35E-04
Total	1.55E-03	1.43E-03	2.22E-01
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for both columns. NE – Not estimated.			

Table 6-47. Rural/Suburban, Non-Smoking: Ethylbenzene Inhalation by Children (mg/kg-day)

Microenvironment	<1 year	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years
<i>Central Tendency</i>						
Home	8.87E-04	8.68E-04	6.16E-04	4.76E-04	3.54E-04	2.72E-04
School ^a	NE	3.15E-05	6.83E-05	7.89E-05	5.88E-05	4.23E-05
Outdoor ^a	1.30E-05	2.06E-05	3.56E-05	2.94E-05	2.19E-05	1.05E-05
Vehicle	5.47E-05	5.75E-05	7.47E-05	6.17E-05	4.60E-05	3.31E-05
Total	9.54E-04	9.78E-04	7.95E-04	6.46E-04	4.81E-04	3.57E-04
<i>Upper-Bound</i>						
Home	2.32E-03	2.26E-03	1.61E-03	1.24E-03	9.24E-04	7.07E-04
School ^a	NE	8.23E-05	1.78E-04	2.06E-04	1.53E-04	1.11E-04
Outdoor ^a	3.40E-05	5.36E-05	9.29E-05	7.67E-05	5.71E-05	2.74E-05
Vehicle	1.43E-04	1.50E-04	1.95E-04	1.61E-04	1.20E-04	8.65E-05
Total	2.50E-03	2.55E-03	2.07E-03	1.68E-03	1.25E-03	9.32E-04
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for both columns. NE – Not estimated.						

Table 6-48. Rural/Suburban, Non-Smoking: Ethylbenzene Inhalation by Adults (mg/kg-day)

Microenvironment	At-Home Parent	Office Worker	Production Worker
Central Tendency			
Home	3.57E-04	2.64E-04	2.64E-04
Office	NE	6.30E-05	NE
Industry ^a	NE	NE	2.21E-02
Outdoor ^a	8.22E-06	8.22E-06	8.22E-06
Vehicle	3.45E-05	3.45E-05	3.45E-05
Total	3.99E-04	3.69E-04	2.24E-02
Upper-Bound			
Home	9.30E-04	6.88E-04	6.88E-04
Office	NE	1.65E-04	NE
Industry ^a	NE	NE	2.21E-01
Outdoor ^a	2.14E-05	2.14E-05	2.14E-05
Vehicle	9.01E-05	9.01E-05	9.01E-05
Total	1.04E-03	9.65E-04	2.22E-01
^a – For these microenvironments no distinction in exposure for smokers and non-smokers was available; therefore, identical numbers are presented for both columns. NE – Not estimated.			

6.7.1.2 Ethylbenzene Chain of Commerce Contribution to Inhalation Intake

Ethylbenzene is naturally occurring in the environment and also arises from many anthropogenic activities and sources. As discussed in Section 5.3.1, the proportion of quantified emissions attributable to the ethylbenzene/styrene production chain of commerce is very small (less than 1% of total emissions when considering the major SIC code categories 28 and 30). In areas with significant vehicular traffic, automotive-related sources of ethylbenzene are the primary determinants of indoor concentrations as well as outdoor concentrations (Section 6.3). Although building materials and household products made of styrene-containing polymers may provide a small contribution to concentrations in indoor air, their contribution to indoor air would be taken into account in the actual indoor air measurements. A number of studies have consistently demonstrated that major indoor sources of ethylbenzene are ETS, attached garages, and household products containing ethylbenzene as part of petroleum mixtures and mixed xylenes. As such, these sources are not part of the ethylbenzene producers' chain of commerce.

Because the chain of commerce contribution to outdoor levels is very small, and that to indoor air has not been quantified but also appears to be very small, the assumption that the chain of commerce contributes about 1% (roughly twice the fractional contribution of industry sectors including the ethylbenzene producers to total NEI estimated emissions in 1999, 0.6%) to total ambient ethylbenzene levels appears to be conservative. Thus, it seems reasonable to estimate that the ethylbenzene/styrene chain of commerce is likely to contribute no more than one one-hundredth of the general public's total inhalation exposure to ethylbenzene.

6.7.2 Dietary Exposure

6.7.2.1 Mother's Milk

As discussed in Section 6.1.3, there are few data available concerning transfer of ethylbenzene into human milk, and hence to infants via breastfeeding. A PBPK model was used (as described in Section 6.1.3) to estimate exposure to ethylbenzene for breastfed children aged 0 to 12 months. Ethylbenzene levels in mother's milk were estimated using the PBPK model and the intake of the breastfed child was then calculated (Sweeney and Gargas, 2006). The estimated mean average daily dose of ethylbenzene for a breastfed infant in the general population was estimated to be 9×10^{-6} mg/kg-day, and the upper-bound was estimated as 2×10^{-5} mg/kg-day. The estimated mean average daily dose of ethylbenzene for the breastfed infant of an occupationally exposed mother was estimated to be 2×10^{-4} mg/kg-day and the upper-bound was 2×10^{-3} mg/kg-day. (Table 6-49).

Table 6-49. Infant Exposure to Ethylbenzene via Breastfeeding

	Predicted Concentration of Ethylbenzene in Mother's Milk (µg/L)	Ingestion of Ethylbenzene via Breastfeeding (mg/kg-d)
General Public		
Central	0.11	9.1E-06
Upper	0.25	2.0E-05
Occupational Exposure		
Central	2.2	1.8E-04
Upper	21.0	1.7E-03

The concentrations of ethylbenzene in ready-to-feed soy- and milk-based infant formulas examined in the FDA's TDS (ranging from 25 to 50 ppb) (Table 6-26) were a hundred times higher than those estimated in mother's milk for the general population and ten times higher than the central tendency estimate in breast milk of occupationally exposed workers. Thus, a young infant ingesting the same volume of these formulas as mother's milk would receive a commensurately higher dose. Thus, although lactational transfer is conservatively modeled here, it does not appear to result in exposures as high as those typical of the U.S. food supply.

6.7.2.2 Total Dietary Intake

Central tendency and upper-bound estimates of total ethylbenzene intake from the diet were calculated for prospective parents and children. These estimates were calculated by combining the mean and maximum concentrations of ethylbenzene measured in FDA market basket surveys for the four-year period 1998 – 2001 (see Table 6-26) with FDA's age group-specific consumption rates for ethylbenzene-containing food items (FDA, 2004b; Appendix M). The food consumption amounts, collectively referred to as the TDS diets, are compiled for the total U.S. population and 14 age/sex subgroups. Because the focus of this exposure assessment is on prospective parents and children, only the lower ten age groups were considered (Table 6-37).

The estimated average and upper-bound intakes, expressed in mg/day for each age group and for each food item individually, are presented in Table 6-50 and Table 6-51, respectively.

Ingestion rates for children and adults are also found in EPA guidance (EPA, 1997a, 2002b); however, the list of foods for which ingestion rates are given is more limited than that provided in the FDA survey and contained fewer product-specific ingestion rates. A direct comparison of the EPA and FDA ingestion rates is difficult because both foods and consumers are characterized differently. For those food categories and age groups that could be compared, some of the ingestion rates in the EPA data were higher than that in the FDA and for other products the opposite was true. The FDA data set, which is designed for detailed evaluation of contemporary U.S. dietary habits and exposures to widespread chemicals, such as ethylbenzene (FDA, 2004a), was considered to be a more robust tool for evaluating potential exposure to children and prospective parents. In particular, the FDA data base also had information on dietary intake from formula for infants less than a year old, which can be compared to the intake from mother's milk.

While one or more food items from each of these categories may be eaten by an individual in a given day, it is unlikely that every food listed in all categories would be consumed daily by an individual. However, because it is not known which and how many of these foods are ingested by an individual in a given day, it was assumed for this screening assessment that total daily intake of ethylbenzene was the sum of the intake from all food items listed. Knowing the total daily dietary intake of ethylbenzene and the total daily intake of food, it is possible to estimate the concentration of ethylbenzene in the total diet for each age group:

$$[\text{Ethylbenzene}] \text{ in Total Diet } \left[\frac{\text{mg}}{\text{kg}} \right] = \frac{\sum \text{Intake}_{\text{EB in food}}}{\sum \text{Intake}_{\text{Food}} \cdot \text{CF}} \quad \{6-14\}$$

where

$\text{Intake}_{\text{EB in food}}$ = Age group-specific total dietary ethylbenzene intake (mg/day; Table 6-50 and Table 6-51)

$\text{Intake}_{\text{Food}}$ = Age group-specific total daily food consumption (g/day) (Table 6-37)

CF = Conversion Factor (1 kg / 1000 g)

Total daily average and upper-bound age group-specific rates of dietary ethylbenzene intake were calculated as:

$$\text{Ethylbenzene intake rate}_{\text{age}} \left[\frac{\text{mg}}{\text{kg-day}} \right] = \frac{\sum \text{Intake}_{\text{EB in food}}}{\text{BW}_{\text{age}}} \quad \{6-15\}$$

where:

$\text{Intake}_{\text{EB in food}}$ = Age group-specific total dietary ethylbenzene intake (mg/day; Table 6-50 and Table 6-51)

BW_{age} = Age group-specific body weight (Table 6-37)

Table 6-50. Estimated Average Age Group-Specific Daily Dietary Intake Rate of Ethylbenzene (mg/day)^a

Category/Food Item Containing Ethylbenzene	Age Group (years)									
	<1 ^b	2	6	10	14-16 (female)	14-16 (male)	25-30 (female)	25-30 (male)	40-45 (female)	40-45 (male)
Dairy										
Cream cheese	4.00E-06	5.30E-05	7.60E-05	1.10E-04	8.60E-05	1.50E-04	1.10E-04	8.50E-05	1.00E-04	8.90E-05
Milk-based infant formula, low iron, ready-to-feed	1.60E-03	NE	NE	NE	NE	NE	NE	NE	NE	NE
Swiss cheese	2.30E-06	3.70E-05	4.20E-05	2.60E-05	3.40E-05	5.40E-05	9.00E-05	1.00E-04	7.50E-05	1.20E-04
Vanilla flavored light ice cream	5.70E-06	7.20E-05	1.20E-04	2.30E-04	2.00E-04	1.50E-04	1.50E-04	9.20E-05	2.50E-04	2.10E-04
Vanilla ice cream	3.30E-05	3.50E-04	7.30E-04	9.00E-04	4.70E-04	8.30E-04	3.60E-04	4.80E-04	4.20E-04	8.80E-04
Whole milk, fluid	2.50E-03	5.50E-03	4.20E-03	3.30E-03	1.90E-03	3.10E-03	1.40E-03	1.70E-03	8.10E-04	1.60E-03
Eggs										
Eggs, scrambled with added milk and fat	6.30E-05	1.60E-04	1.00E-04	1.10E-04	8.40E-05	1.30E-04	1.40E-04	2.70E-04	1.10E-04	1.40E-04
Fruits and Vegetables										
Soy-based infant formula, ready-to-feed	4.80E-03	NE	NE	NE	NE	NE	NE	NE	NE	NE
Apple, red with peel, raw	5.40E-05	7.30E-04	7.80E-04	6.10E-04	4.10E-04	4.00E-04	3.70E-04	5.80E-04	5.70E-04	4.80E-04
Meat										
Beef, ground, regular hamburger, cooked in patty shape	1.90E-06	1.00E-05	2.00E-05	2.10E-05	2.40E-05	4.70E-05	1.70E-05	3.20E-05	1.90E-05	3.00E-05
Bologna	2.00E-06	5.90E-06	6.50E-06	9.70E-06	5.60E-06	9.70E-06	3.80E-06	1.20E-05	4.60E-06	1.20E-05
Chicken nuggets, fast-food	4.30E-06	4.60E-05	6.90E-05	5.60E-05	4.30E-05	4.50E-05	2.50E-05	2.90E-05	1.80E-05	1.50E-05
Chicken, fried (breast, leg, thigh), fast-food	1.50E-07	1.60E-05	6.90E-06	1.20E-05	4.20E-05	4.80E-05	2.30E-05	4.80E-05	3.40E-05	4.50E-05
Frankfurters, (beef/beef and pork), boiled	3.60E-06	2.80E-05	2.70E-05	2.00E-05	1.10E-05	1.90E-05	1.10E-05	2.10E-05	1.30E-05	2.00E-05
Meatloaf, beef, homemade	7.80E-06	1.80E-05	1.80E-05	1.40E-05	6.30E-05	4.10E-05	2.30E-05	3.50E-05	3.00E-05	8.00E-05
Pork, bacon, oven cooked	1.00E-07	1.90E-06	1.80E-06	2.50E-06	2.20E-06	5.30E-06	2.70E-06	5.20E-06	2.70E-06	5.00E-06
Quarter-pound cheeseburger on bun, fast-food	1.60E-06	1.10E-05	1.90E-05	2.90E-05	4.20E-05	7.10E-05	3.20E-05	1.10E-04	1.80E-05	4.10E-05
Quarter-pound hamburger sandwich on white roll with garnish, fast-food type	7.00E-07	2.10E-05	4.90E-05	7.80E-05	4.50E-05	8.60E-05	4.00E-05	1.30E-04	5.90E-05	1.10E-04
Salami, lunch meat type, regular, not hard	1.90E-07	4.00E-06	6.90E-06	7.00E-06	5.30E-06	1.50E-05	6.00E-06	1.10E-05	5.20E-06	1.40E-05
Taco/tostada, from Mexican carry-out	3.80E-07	9.50E-06	2.50E-05	5.60E-05	6.30E-05	7.40E-05	5.00E-05	9.20E-05	4.60E-05	3.70E-05
Fish										
Fish sticks, commercial, frozen, oven cooked	1.80E-06	9.70E-06	1.50E-05	3.00E-06	1.70E-05	1.00E-05	8.80E-06	1.40E-05	8.50E-06	5.50E-06
Tuna, canned in oil, drained	5.50E-06	3.00E-05	2.90E-05	8.70E-05	7.60E-05	4.60E-05	7.70E-05	7.60E-05	8.20E-05	9.20E-05
Fast food										
Cheese and pepperoni pizza, regular crust, from pizza carry-out	1.70E-06	1.60E-05	3.70E-05	4.20E-05	5.10E-05	1.10E-04	3.70E-05	8.90E-05	3.50E-05	3.90E-05

Category/Food Item Containing Ethylbenzene	Age Group (years)									
	<1 ^b	2	6	10	14-16 (female)	14-16 (male)	25-30 (female)	25-30 (male)	40-45 (female)	40-45 (male)
Cheese pizza, regular crust, from pizza carry-out	2.60E-06	4.10E-05	8.10E-05	1.10E-04	8.70E-05	1.90E-04	7.30E-05	8.10E-05	3.30E-05	5.40E-05
French fries, fast food	9.90E-06	7.30E-05	9.00E-05	1.10E-04	1.30E-04	1.90E-04	9.50E-05	2.40E-04	6.20E-05	8.60E-05
Potato chips, commercial	7.40E-07	4.70E-05	6.90E-05	7.10E-05	5.70E-05	1.00E-04	5.90E-05	1.30E-04	4.90E-05	7.80E-05
Fats										
Butter, regular (salted)	2.20E-06	1.00E-05	1.80E-05	1.30E-05	8.90E-06	2.40E-05	1.70E-05	2.70E-05	2.10E-05	3.10E-05
Margarine, stick, regular (salted)	3.30E-06	1.60E-05	2.30E-05	2.90E-05	2.20E-05	3.90E-05	2.20E-05	2.70E-05	3.30E-05	4.10E-05
Olive/safflower oil	4.10E-07	6.20E-07	3.90E-06	2.10E-07	6.20E-07	8.30E-07	1.70E-06	1.10E-05	2.50E-06	2.10E-06
Grains										
Apple pie fresh/frozen, commercial	3.30E-07	1.80E-06	1.10E-05	2.80E-06	8.30E-06	1.80E-05	1.80E-05	1.00E-05	3.00E-05	4.00E-05
Brownies, commercial	NE	2.90E-06	1.70E-05	1.10E-05	1.60E-05	1.50E-05	6.20E-06	4.10E-06	8.10E-06	1.70E-05
Butter-type crackers (e.g., ritz, hi-ho)	2.20E-05	8.10E-05	7.50E-05	5.50E-05	6.00E-05	6.70E-05	7.60E-05	5.30E-05	5.90E-05	6.10E-05
Cake doughnuts with icing, any flavor, from doughnut store	6.40E-07	8.10E-06	1.40E-05	1.50E-05	2.90E-05	3.00E-05	1.20E-05	2.00E-05	1.30E-05	2.20E-05
Chocolate cake with chocolate icing, commercial	1.10E-06	6.50E-06	1.90E-05	3.10E-05	1.10E-05	2.00E-05	2.90E-05	1.80E-05	1.70E-05	1.70E-05
Chocolate chip cookies, commercial	7.00E-06	7.00E-05	1.10E-04	1.20E-04	9.10E-05	1.40E-04	6.60E-05	8.90E-05	5.70E-05	9.10E-05
Corn chips	1.30E-06	7.60E-06	1.20E-05	1.80E-05	2.70E-05	2.80E-05	1.50E-05	3.30E-05	9.10E-06	1.40E-05
Graham crackers	4.10E-05	5.50E-05	5.50E-05	1.90E-05	2.40E-05	2.70E-05	1.20E-05	1.50E-05	2.90E-05	1.30E-05
Muffins (blueberry/plain)	1.00E-05	1.70E-04	2.90E-04	3.20E-04	2.20E-04	3.40E-04	1.60E-04	2.20E-04	2.30E-04	2.60E-04
Popcorn, popped in oil	1.50E-07	3.40E-06	5.90E-06	8.60E-06	3.40E-06	7.20E-06	6.10E-06	8.00E-06	6.80E-06	6.20E-06
Sandwich cookies with cream filling, commercial	4.80E-06	5.50E-05	7.80E-05	8.80E-05	6.40E-05	9.90E-05	7.00E-05	5.00E-05	4.00E-05	6.80E-05
Sugar cookies, commercial	3.90E-06	1.80E-05	2.20E-05	2.00E-05	1.40E-05	1.60E-05	1.40E-05	1.60E-05	1.50E-05	1.30E-05
Sweet roll/Danish, commercial	7.70E-07	3.70E-06	8.10E-06	8.50E-06	1.40E-05	2.50E-05	9.70E-06	1.10E-05	1.40E-05	1.50E-05
White bread, enriched	9.00E-05	4.90E-04	7.10E-04	7.80E-04	6.70E-04	7.00E-04	5.70E-04	8.90E-04	6.50E-04	9.70E-04
Nuts										
Mixed nuts, no peanuts, dry roasted	NE	3.80E-06	4.80E-06	5.00E-06	4.50E-06	2.40E-06	1.20E-05	1.50E-05	8.50E-06	1.70E-05
Peanut butter, creamy, commercial in jar	1.80E-06	2.70E-05	3.80E-05	2.90E-05	1.60E-05	2.70E-05	8.40E-06	1.70E-05	6.90E-06	1.90E-05
Sweets										
Candy, caramels	3.10E-07	1.20E-05	1.90E-05	5.00E-05	2.00E-05	4.30E-05	2.20E-05	2.90E-05	2.40E-05	2.50E-05
Milk chocolate candy bar, plain	1.70E-07	1.70E-05	2.70E-05	3.20E-05	3.90E-05	5.10E-05	3.90E-05	3.10E-05	3.60E-05	5.50E-05
TOTAL	9.30E-03 2.90E-03^c	8.40E-03	8.20E-03	7.70E-03	5.30E-03	7.70E-03	4.30E-03	6.10E-03	4.20E-03	6.10E-03
^a Calculated using mean food concentration data from Table 6-26 and FDA food consumption data (Appendix M) ^b Aged 6 to 11 months ^c Total excluding intake of infant formula. NE – Not estimated.										

Table 6-51. Estimated Age Group-Specific Upper-Bound Daily Dietary Intake Rate of Ethylbenzene (mg/day)^a

Category/Food Item Containing Ethylbenzene	Age Group (years)									
	<1 ^b	2	6	10	14-16 (female)	14-16 (male)	25-30 (female)	25-30 (male)	40-45 (female)	40-45 (male)
Dairy										
Cream cheese	8.00E-06	1.10E-04	1.50E-04	2.10E-04	1.70E-04	3.10E-04	2.20E-04	1.70E-04	2.10E-04	1.80E-04
Milk-based infant formula, low iron, ready-to-feed	2.60E-03	NE	NE	NE	NE	NE	NE	NE	NE	NE
Swiss cheese	3.00E-06	5.00E-05	5.60E-05	3.40E-05	4.60E-05	7.20E-05	1.20E-04	1.40E-04	1.00E-04	1.60E-04
Vanilla flavored light ice cream	1.00E-05	1.30E-04	2.20E-04	4.00E-04	3.40E-04	2.60E-04	2.70E-04	1.60E-04	4.30E-04	3.70E-04
Vanilla ice cream	4.00E-05	4.20E-04	8.80E-04	1.10E-03	5.70E-04	9.90E-04	4.30E-04	5.80E-04	5.00E-04	1.10E-03
Whole milk, fluid	4.20E-03	9.00E-03	7.00E-03	5.40E-03	3.10E-03	5.20E-03	2.20E-03	2.90E-03	1.30E-03	2.70E-03
Eggs										
Eggs, scrambled with added milk and fat	1.40E-04	3.70E-04	2.30E-04	2.50E-04	1.90E-04	3.00E-04	3.20E-04	6.10E-04	2.50E-04	3.10E-04
Fruits and Vegetables										
Soy-based infant formula, ready-to-feed	6.40E-03	NE	NE	NE	NE	NE	NE	NE	NE	NE
Apple, red with peel, raw	6.90E-05	9.30E-04	9.90E-04	7.70E-04	5.30E-04	5.10E-04	4.70E-04	7.40E-04	7.30E-04	6.10E-04
Meat										
Beef, ground, regular hamburger, cooked in patty shape	2.50E-06	1.30E-05	2.60E-05	2.70E-05	3.10E-05	6.00E-05	2.20E-05	4.20E-05	2.40E-05	3.90E-05
Bologna	2.70E-06	7.80E-06	8.60E-06	1.30E-05	7.40E-06	1.30E-05	5.00E-06	1.60E-05	6.20E-06	1.60E-05
Chicken nuggets, fast-food	2.70E-05	2.90E-04	4.30E-04	3.50E-04	2.70E-04	2.80E-04	1.50E-04	1.80E-04	1.10E-04	9.10E-05
Chicken, fried (breast, leg, thigh), fast-food	5.00E-07	5.40E-05	2.40E-05	4.20E-05	1.40E-04	1.70E-04	7.80E-05	1.70E-04	1.20E-04	1.50E-04
Frankfurters, (beef/beef and pork), boiled	5.90E-06	4.60E-05	4.40E-05	3.30E-05	1.80E-05	3.20E-05	1.70E-05	3.50E-05	2.10E-05	3.20E-05
Meatloaf, beef, homemade	2.00E-05	4.80E-05	4.80E-05	3.70E-05	1.60E-04	1.10E-04	5.90E-05	9.00E-05	7.70E-05	2.10E-04
Pork, bacon, oven cooked	1.20E-07	2.30E-06	2.20E-06	3.00E-06	2.60E-06	6.40E-06	3.30E-06	6.30E-06	3.20E-06	6.00E-06
Quarter-pound cheeseburger on bun, fast-food	4.50E-06	3.10E-05	5.40E-05	8.20E-05	1.20E-04	2.00E-04	9.20E-05	3.10E-04	5.20E-05	1.20E-04
Quarter-pound hamburger sandwich on white roll with garnish, fast-food type	2.30E-06	6.70E-05	1.60E-04	2.50E-04	1.50E-04	2.80E-04	1.30E-04	4.10E-04	1.90E-04	3.50E-04
Salami, lunch meat type, regular, not hard	4.80E-07	1.00E-05	1.70E-05	1.80E-05	1.30E-05	3.90E-05	1.50E-05	2.60E-05	1.30E-05	3.60E-05
Taco/tostada, from Mexican carry-out	8.00E-07	2.00E-05	5.20E-05	1.20E-04	1.30E-04	1.50E-04	1.10E-04	1.90E-04	9.60E-05	7.70E-05
Fish										
Fish sticks, commercial, frozen, oven cooked	6.50E-06	3.40E-05	5.20E-05	1.10E-05	6.00E-05	3.50E-05	3.10E-05	4.80E-05	3.00E-05	2.00E-05
Tuna, canned in oil, drained	1.10E-05	5.70E-05	5.50E-05	1.70E-04	1.50E-04	8.80E-05	1.50E-04	1.50E-04	1.60E-04	1.80E-04
Fast food										
Cheese and pepperoni pizza, regular crust, from pizza carry-out	2.80E-06	2.70E-05	6.20E-05	7.00E-05	8.50E-05	1.90E-04	6.20E-05	1.50E-04	5.90E-05	6.50E-05

Category/Food Item Containing Ethylbenzene	Age Group (years)									
	<1 ^b	2	6	10	14-16 (female)	14-16 (male)	25-30 (female)	25-30 (male)	40-45 (female)	40-45 (male)
Cheese pizza, regular crust, from pizza carry-out	1.10E-05	1.60E-04	3.20E-04	4.30E-04	3.40E-04	7.50E-04	2.90E-04	3.20E-04	1.30E-04	2.10E-04
French fries, fast food	4.80E-05	3.50E-04	4.30E-04	5.50E-04	6.20E-04	9.20E-04	4.60E-04	1.10E-03	3.00E-04	4.20E-04
Potato chips, commercial	1.30E-06	8.30E-05	1.20E-04	1.30E-04	1.00E-04	1.80E-04	1.00E-04	2.20E-04	8.60E-05	1.40E-04
Fats										
Butter, regular (salted)	6.00E-06	2.90E-05	5.10E-05	3.70E-05	2.50E-05	6.60E-05	4.60E-05	7.50E-05	5.90E-05	8.60E-05
Margarine, stick, regular (salted)	1.20E-05	6.00E-05	8.40E-05	1.10E-04	8.20E-05	1.40E-04	8.30E-05	1.00E-04	1.20E-04	1.50E-04
Olive/safflower oil	1.00E-06	1.50E-06	9.50E-06	5.00E-07	1.50E-06	2.00E-06	4.00E-06	2.60E-05	6.00E-06	5.00E-06
Grains										
Apple pie fresh/frozen, commercial	7.00E-07	3.80E-06	2.40E-05	5.90E-06	1.80E-05	3.80E-05	3.70E-05	2.20E-05	6.30E-05	8.40E-05
Brownies, commercial	NE	8.50E-06	5.10E-05	3.30E-05	4.70E-05	4.50E-05	1.80E-05	1.20E-05	2.40E-05	5.20E-05
Butter-type crackers (e.g., ritz, hi-ho)	7.90E-05	2.90E-04	2.70E-04	2.00E-04	2.10E-04	2.40E-04	2.70E-04	1.90E-04	2.10E-04	2.20E-04
Cake doughnuts with icing, any flavor, from doughnut store	1.00E-06	1.30E-05	2.30E-05	2.40E-05	4.80E-05	4.90E-05	2.00E-05	3.20E-05	2.10E-05	3.60E-05
Chocolate cake with chocolate icing, commercial	7.00E-06	4.00E-05	1.20E-04	1.90E-04	6.80E-05	1.20E-04	1.80E-04	1.10E-04	1.00E-04	1.10E-04
Chocolate chip cookies, commercial	2.40E-05	2.40E-04	3.60E-04	4.10E-04	3.20E-04	4.70E-04	2.30E-04	3.10E-04	2.00E-04	3.10E-04
Corn chips	1.70E-06	9.80E-06	1.50E-05	2.40E-05	3.50E-05	3.60E-05	1.90E-05	4.20E-05	1.20E-05	1.80E-05
Graham crackers	1.40E-04	1.80E-04	1.80E-04	6.30E-05	7.90E-05	8.80E-05	3.80E-05	4.90E-05	9.40E-05	4.40E-05
Muffins (blueberry/plain)	5.40E-05	8.80E-04	1.50E-03	1.60E-03	1.10E-03	1.70E-03	8.10E-04	1.20E-03	1.20E-03	1.30E-03
Popcorn, popped in oil	2.00E-07	4.50E-06	7.80E-06	1.10E-05	4.60E-06	9.60E-06	8.10E-06	1.10E-05	9.10E-06	8.30E-06
Sandwich cookies with cream filling, commercial	9.00E-06	1.00E-04	1.50E-04	1.70E-04	1.20E-04	1.90E-04	1.30E-04	9.40E-05	7.50E-05	1.30E-04
Sugar cookies, commercial	1.40E-05	6.50E-05	7.60E-05	7.10E-05	5.10E-05	5.60E-05	5.10E-05	5.70E-05	5.50E-05	4.70E-05
Sweet roll/Danish, commercial	1.20E-06	5.60E-06	1.20E-05	1.30E-05	2.10E-05	3.70E-05	1.50E-05	1.60E-05	2.10E-05	2.20E-05
White bread, enriched	2.70E-04	1.50E-03	2.10E-03	2.30E-03	2.00E-03	2.10E-03	1.70E-03	2.70E-03	1.90E-03	2.90E-03
Nuts										
Mixed nuts, no peanuts, dry roasted	NE	1.10E-05	1.40E-05	1.50E-05	1.30E-05	7.20E-06	3.50E-05	4.60E-05	2.50E-05	5.10E-05
Peanut butter, creamy, commercial in jar	9.50E-06	1.40E-04	2.00E-04	1.50E-04	8.40E-05	1.50E-04	4.50E-05	9.10E-05	3.70E-05	1.00E-04
Sweets										
Candy, caramels	1.00E-06	4.00E-05	6.20E-05	1.60E-04	6.50E-05	1.40E-04	6.90E-05	9.20E-05	7.60E-05	7.90E-05
Milk chocolate candy bar, plain	1.00E-06	9.50E-05	1.60E-04	1.80E-04	2.30E-04	2.90E-04	2.20E-04	1.80E-04	2.10E-04	3.20E-04
TOTAL	1.40E-02 5.00E-03^c	1.60E-02	1.70E-02	1.60E-02	1.20E-02	1.70E-02	9.90E-03	1.40E-02	9.60E-03	1.40E-02
^a Calculated using maximum food concentration data from Table 6-26 and FDA food consumption data (Appendix M) ^b Aged 6 to 11 months ^c Total excluding intake of infant formula. NE – Not estimated.										

The resulting average and upper-bound estimates of total dietary ethylbenzene concentrations and total dietary intakes of ethylbenzene for each age group are presented in Table 6-52.

Table 6-52. Average and Upper-Bound Estimates of Ethylbenzene Concentration in the Total Diet (µg/kg), and Daily Intake Rate (mg/kg-day)

Exposure Level	Age Group						
	<1 ^a	2	6	10	14-16	25-30	40-45
Central Tendency							
[EB] in food	8.04E+00	6.37E+00	5.53E+00	4.58E+00	3.12E+00	2.25E+00	2.14E+00
EB Intake Rate	9.90E-04 3.09E-04	6.49E-04	3.78E-04	2.15E-04	1.03E-04	7.29E-05	6.94E-05
Upper-Bound							
[EB] in food	1.23E+01	1.22E+01	1.14E+01	9.77E+00	6.99E+00	5.18E+00	4.84E+00
EB Intake Rate	1.51E-03 5.32E-04	1.25E-03	7.77E-04	4.59E-04	2.31E-04	1.68E-04	1.57E-04

^a Aged six to 11 months. First number under intake is for non-breastfeeding infant with second number intake for breastfeeding infant (excluding intake of infant formula).

As shown in Table 6-52, infants less than one year old had the highest estimated total daily ethylbenzene intake. The majority of this intake was from ingestion of infant formula or milk (Figure 6-8). Soy-based formula alone accounted for around 52% of the total average ethylbenzene intake in a day.

In all the other age groups examined, the top four contributing foods are the same: whole milk, white bread, blueberry muffins, and unpeeled red apple. These items are both relatively high in ethylbenzene concentration (Table 6-26) and relatively highly consumed (Appendix M). It is noted that, as discussed in Section 6.4, dietary concentrations of ethylbenzene are generally very low, as are these calculated intakes.

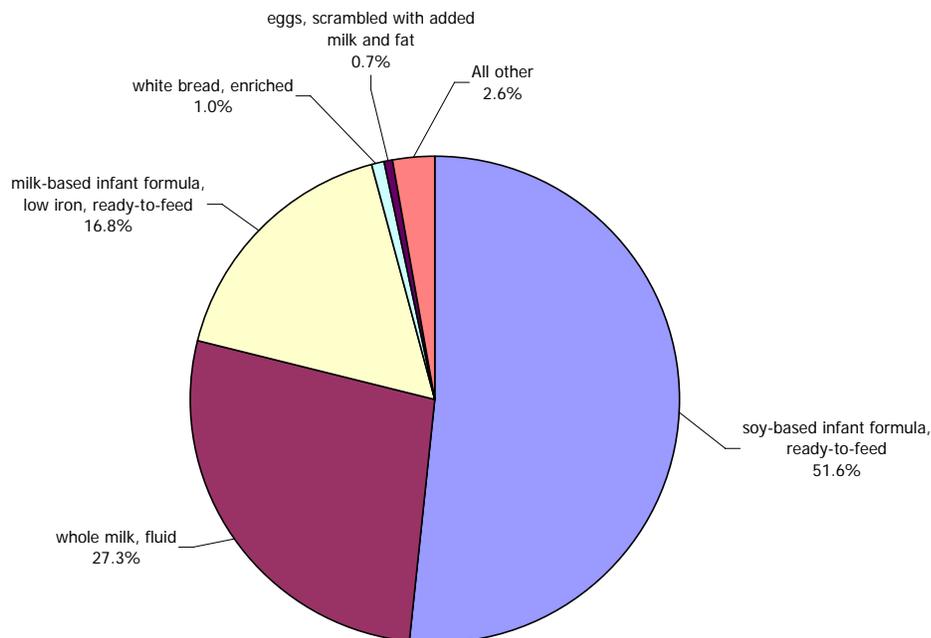


Figure 6-8. Contribution of Individual Food Items to Total Average Dietary Intake of Ethylbenzene in Children Aged 6 to 11 Months

6.7.2.3 Chain of Commerce Contribution to Dietary Intake

As discussed in Section 6.4.3.2, kinetic modeling was used to derive a more realistic estimate of total dietary ethylbenzene concentration (0.45 µg/kg) due to migration. In order to estimate the migration-derived proportion of ethylbenzene in the food consumed by the identified populations, these values can be compared to the estimated central tendency and upper-bound age group-specific dietary concentrations calculated according to Equation {6-15} and presented in Table 6-52. The results of these comparisons are presented in Table 6-53.

Table 6-53. Estimated Percent Contribution of Styrenic Food-Contact Materials to Estimated Age Group-Specific Central Tendency^a and Upper-Bound^b Total Dietary Concentrations of Ethylbenzene

	Age Group						
	<1	2	6	10	14-16	25-30	40-45
<i>Kinetic Modeling Estimate (0.45 µg/kg)^c</i>							
Central	6%	7%	8%	10%	14%	20%	21%
Upper	4%	4%	4%	5%	6%	9%	9%
^a Values presented in Table 6-50							
^b Values presented in Table 6-51							
^c See Section 6.4.3.2							

Estimates of contribution derived from kinetic modeling (Section 6.4.3.2), ranged from approximately 6% to 21% (average case) and 4% to 9% (upper-bound case). A conservative assumption based on these results is that 15 - 25% of ethylbenzene in the U.S. diet has migrated from food-contact materials. Thus, the chain of commerce contribution to ethylbenzene dietary intake in this exposure assessment is one-fourth (25%) of the calculated intakes.

6.7.3 Exposure to Children from Mouthing Toys

As discussed in Section 6.5, commercial styrene-containing polymers are used in the manufacturing of children's toys, such as balls, playmats, and gym sets. Although toys designed for mouthing by young children, such as teething rings, are typically made from polyvinyl chloride (PVC) (Steiner *et al.*, 1998) rather than styrene-containing polymers, it was conservatively assumed that all non-pacifier objects mouthed by young children are made of styrene-containing polymers. Assuming that the migration rate of ethylbenzene from toys does not decrease over the age interval for which exposure is estimated (*i.e.*, mass balance is ignored after the toy is purchased), an estimated DMR of ethylbenzene from a toy or object that is two months old was determined to be 0.0002 $\mu\text{g}/\text{cm}^2\text{-day}$ (see Section 6.5).

Using the estimated DMR of ethylbenzene from a toy and conservatively assuming that all ethylbenzene migrating from the object during contact is ingested by the child, an upper-bound value for intake, that likely exceeds any such exposures in reality, can be estimated as described in the following sections.

6.7.3.1 Exposure Frequency and Duration

To determine the potential daily exposure to a child, a key variable is the time of contact of the child's mouth with the object. According to the U.S. Consumer Product Safety Commission (CPSC) Chronic Health Advisory Panel, children's mouthing behavior tends to decline rapidly after age three (USCPSC, 1998, EPA, 2002b). Of three recent studies that have evaluated mouthing behavior by young children (Juberg *et al.*, 2001; Groot *et al.*, 1998; Zartarian *et al.*, 1997), only that by Juberg *et al.* (2001) was not included in EPA's *Child-Specific Exposure Factors Handbook* (EPA, 2002b) (presumably because of its publication date). However, as the most extensive study of mouthing habits yet published, and because it was relied upon by the CPSC in its studies of potential exposure of children to phthalates via mouthing, Juberg *et al.*'s (2001) results were used. Juberg *et al.*'s (2001) daily mouthing time estimates are quite similar to those recommended in guidance.

The Juberg *et al.* (2001) study was conducted in three phases, the first of which was a pilot phase that examined groups of 15 children aged 0 - 18 months or 19 - 36 months. In this phase, parents observed their children for an entire day in a normal environment and documented each item mouthed and its time in and out of the child's mouth. The second phase involved a larger sample with groups of 92 - 95 children. The final phase focused on children 3 - 18 months of age ($n = 168$), with parents observing the children for non-pacifier mouthing behavior (including non-plastic objects) for five consecutive days. The results from all three phases indicated that children aged 0 - 18 months mouth non-pacifier objects, on average, approximately 35 minutes per day, with a maximum time of 325 - 350 minutes per day. A significant decrease in mouthing was observed in the 19- to 36-month age group,

who exhibited a mean of approximately two minutes per day with a maximum of 200 - 220 minutes per day.

Based on these results, children aged 2 – 12 and 13 - 24 months were conservatively assumed to mouth ethylbenzene-containing polymers for an average of 35 minutes per day, with a maximum of 350 minutes per day. For children aged 25-36 months, contact was assumed to be on average 2 minutes per day, with a maximum of 220 minutes per day. Insignificant toy mouthing activity was assumed for children older than 36 months.

6.7.3.2 Contact Assumptions

The body surface area assumed to come in contact with the polystyrene-containing object is the entire inside of the child's mouth. The surface area of the oral cavity of an adult has been estimated to be 102 cm², based on information provided in Cheng *et al.* (1997) and Collins and Dawes (1987). Scaling by body weight^{2/3} (surface area scaling – [infant BW/adult BW]^{2/3}), the estimated surface area for the oral cavity of the 2 - 12 month old, 1 - 2 and 2 -, 3-year old would be 24.4, 31.1, and 34.1 cm², respectively (mean body weights for male and female children between the ages of 2 months and 3 years [EPA, 2002b]; default adult body weight = 71.8 kg [EPA, 1997a]). These values are consistent with the relationship between the surface area for the root (base) of the tongue in the infant (5 cm²) to the adult (17 cm²) reported in ICRP (1975). Use of these values assumes that all of the ethylbenzene that migrates from the object to the saliva during the mouthing activity is ingested.

6.7.3.3 Calculation of Age-Specific Exposure Rates

Adjusting for the time of mouthing activity per day and the child's body weight, the following equation was used to estimate daily exposure for each age group:

$$\text{Ethylbenzene Intake} \left[\frac{\text{mg}}{\text{kg-day}} \right] = \frac{\text{DMR} \times \text{ET} \times \text{SA}_{\text{oral}} \times 0.001 \frac{\text{mg}}{\mu\text{g}}}{1,440 \frac{\text{min}}{\text{day}} \times \text{BW}} \quad \{6-16\}$$

where

- DMR = Daily migration rate of ethylbenzene from the toy or object when the object is two months old (μg/cm²-day) (from Equation {6-12})
- ET = age-specific time of mouthing activity (minutes/day)
- SA_{oral} = age-specific surface area of the oral cavity (cm²)
- BW = age interval-specific body weight (kg)

Using this equation, the mean and maximum estimated daily exposures to ethylbenzene from mouthing of styrenic toys is provided in Table 6-54.

Table 6-54. Estimated Ethylbenzene Intake in Young Children from Mouthing of Styrenic Toys (mg/kg-day)

Age Group (months)	Mean Body Weight (kg)	Mouthing Time (minutes/day)	Oral Surface Area (cm ²)	Ethylbenzene Intake (mg/kg-day)	
		Average (Maximum)		Average	Maximum
2 – 12	8.5	35 (350)	24.4	1.4E-08	1.4E-07
13 - 24	12.2		31.0	1.3E-08	1.3E-07
25 - 36	14	2 (220)	34.1	6.8E-10	7.5E-08

These daily exposure levels are orders of magnitude lower than those associated with other exposure pathways. It is therefore concluded that mouthing of styrenic toys is unlikely to be a significant source of children’s exposure to ethylbenzene.

6.7.4 Summary

In this section, calculated ethylbenzene exposures via inhalation and ingestion are summed in order to: (1) estimate total exposure for the populations of interest; (2) determine the relative contribution of different sources; and, (3) identify any notable relationships of exposure patterns with age. In order to sum age group-specific inhalation and dietary intakes, the following equivalences were assumed:

Dietary Age Group	Inhalation Age Group
<1 (6 to 11 months)	<1
2	1-2
6	3-5
10	6-8
14-16	Average of 9-14, 15-19
Average of 25-30, 40-45	20 - 45

6.7.4.1 Total Intake

Child Receptors

Table 6-55 to Table 6-58 summarize the estimated ethylbenzene intakes via inhalation, ingestion, and mouthing by children of the different age groups in each exposure setting. The highest intakes were associated with the Urban exposure settings, and inhalation was the dominant exposure route for all populations and exposure settings. While intake from diet was consistent between the various scenarios for each specific receptor and age group, the proportion contributed by diet was higher in the Rural/Suburban, Non-Smoking setting due to less contribution to the total from the inhalation pathway. The total exposure estimates among scenarios and exposure levels differed by only a factor of around two.

Table 6-55. Total Ethylbenzene Intake: Sum of Inhalation and Ingestion Exposures for the Urban, Smoking Setting (mg/kg-day)

Pathway	<1 year (Bottle-fed)		<1 year (Breastfed)		Worker's Child (Breastfed)		1-2 years		3-5 years		6-8 years		9-14 years		15-19 years	
	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total
<i>Central Tendency</i>																
Inhalation	2.64E-03	73%	2.64E-03	89%	2.64E-03	84%	2.64E-03	80%	2.07E-03	85%	1.65E-03	88%	1.22E-03	92%	9.17E-04	90%
Mouthing	1.41E-08	<1%	1.41E-08	<1%	1.41E-08	<1%	1.25E-08	<1%	6.83E-10	<1%						
Breastfeeding			9.10E-06	<1%	1.80E-04	6%										
Total Diet	9.90E-04	27%	3.09E-04	10%	3.09E-04	10%	6.49E-04	20%	3.78E-04	15%	2.15E-04	12%	1.03E-04	8%	1.03E-04	10%
Total	3.63E-03	100%	2.96E-03	100%	3.13E-03	100%	3.29E-03	100%	2.45E-03	100%	1.87E-03	100%	1.32E-03	100%	1.02E-03	100%
<i>Upper-Bound</i>																
Inhalation	5.87E-03	80%	5.87E-03	91%	5.87E-03	72%	5.89E-03	82%	4.62E-03	86%	3.67E-03	89%	2.74E-03	92%	2.05E-03	90%
Mouthing	1.41E-07	<1%	1.41E-07	<1%	1.41E-07	<1%	1.25E-07	<1%	7.51E-08	<1%						
Breastfeeding			2.00E-05	<1%	1.70E-03	21%										
Total Diet	1.51E-03	20%	5.32E-04	8%	5.32E-04	7%	1.25E-03	18%	7.77E-04	14%	4.59E-04	11%	2.31E-04	8%	2.31E-04	10%
Total	7.38E-03	100%	6.42E-03	100%	8.10E-03	100%	7.14E-03	100%	5.40E-03	100%	4.13E-03	100%	2.97E-03	100%	2.28E-03	100%

Table 6-56. Total Ethylbenzene Intake: Sum of Inhalation and Ingestion Exposures for the Urban, Non-Smoking Setting (mg/kg-day)

Pathway	<1 year (Bottle-fed)		<1 year (Breastfed)		Worker's Child (Breastfed)		1-2 years		3-5 years		6-8 years		9-14 years		15-19 years	
	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total
<i>Central Tendency</i>																
Inhalation	1.76E-03	64%	1.76E-03	85%	1.76E-03	78%	1.79E-03	73%	1.43E-03	79%	1.15E-03	84%	8.60E-04	89%	6.38E-04	86%
Mouthing	1.41E-08	<1%	1.41E-08	<1%	1.41E-08	<1%	1.25E-08	<1%	6.83E-10	<1%						
Breastfeeding			9.10E-06	<1%	1.80E-04	8%										
Total Diet	9.90E-04	36%	3.09E-04	15%	3.09E-04	14%	6.49E-04	27%	3.78E-04	21%	2.15E-04	16%	1.03E-04	11%	1.03E-04	14%
Total	2.75E-03	100%	2.08E-03	100%	2.25E-03	100%	2.44E-03	100%	1.81E-03	100%	1.37E-03	100%	9.63E-04	100%	7.41E-04	100%
<i>Upper-Bound</i>																
Inhalation	3.94E-03	72%	3.94E-03	88%	3.94E-03	64%	3.99E-03	76%	3.19E-03	80%	2.57E-03	85%	1.91E-03	89%	1.42E-03	86%
Mouthing	1.41E-07	<1%	1.41E-07	<1%	1.41E-07	<1%	1.25E-07	<1%	7.51E-08	<1%						
Breastfeeding			2.00E-05	<1%	1.70E-03	28%										
Total Diet	1.51E-03	28%	5.32E-04	12%	5.32E-04	9%	1.25E-03	24%	7.77E-04	20%	4.59E-04	15%	2.31E-04	11%	2.31E-04	14%
Total	5.45E-03	100%	4.49E-03	100%	6.17E-03	100%	5.24E-03	100%	3.97E-03	100%	3.03E-03	100%	2.14E-03	100%	1.65E-03	100%

Table 6-57. Total Ethylbenzene Intake: Sum of Inhalation and Ingestion Exposures for the Rural/Suburban, Smoking Setting (mg/kg-day)

Pathway	<1 year (Bottle-fed)		<1 year (Breastfed)		Worker's Child (Breastfed)		1-2 years		3-5 years		6-8 years		9-14 years		15-19 years	
	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total
<i>Central Tendency</i>																
Inhalation	1.43E-03	59%	1.43E-03	82%	1.43E-03	75%	1.44E-03	69%	1.14E-03	75%	9.14E-04	81%	6.81E-04	87%	5.10E-04	83%
Mouthing	1.41E-08	<1%	1.41E-08	<1%	1.41E-08	<1%	1.25E-08	<1%	6.83E-10	<1%						
Breastfeeding			9.10E-06	<1%	1.80E-04	9%										
Total Diet	9.90E-04	41%	3.09E-04	18%	3.09E-04	16%	6.49E-04	31%	3.78E-04	25%	2.15E-04	19%	1.03E-04	13%	1.03E-04	17%
Total	2.42E-03	100%	1.75E-03	100%	1.92E-03	100%	2.09E-03	100%	1.52E-03	100%	1.13E-03	100%	7.84E-04	100%	6.13E-04	100%
<i>Upper-Bound</i>																
Inhalation	3.73E-03	71%	3.73E-03	87%	3.73E-03	63%	3.75E-03	75%	2.98E-03	79%	2.38E-03	84%	1.79E-03	89%	1.33E-03	85%
Mouthing	1.41E-07	<1%	1.41E-07	<1%	1.41E-07	<1%	1.25E-07	<1%	7.51E-08	<1%						
Breastfeeding			2.00E-05	<1%	1.70E-03	29%										
Total Diet	1.51E-03	29%	5.32E-04	12%	5.32E-04	9%	1.25E-03	25%	7.77E-04	21%	4.59E-04	16%	2.31E-04	11%	2.31E-04	15%
Total	5.24E-03	100%	4.28E-03	100%	5.96E-03	100%	5.00E-03	100%	3.76E-03	100%	2.84E-03	100%	2.02E-03	100%	1.56E-03	100%

Table 6-58. Total Ethylbenzene Intake: Sum of Inhalation and Ingestion Exposures for the Rural/Suburban, Non-Smoking Setting (mg/kg-day)

Pathway	<1 year (Bottle-fed)		<1 year (Breastfed)		Worker's Child (Breastfed)		1-2 years		3-5 years		6-8 years		9-14 years		15-19 years	
	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total	Intake	% of Total
<i>Central Tendency</i>																
Inhalation	9.54E-04	49%	9.54E-04	75%	9.54E-04	66%	9.78E-04	60%	7.95E-04	68%	6.46E-04	75%	4.81E-04	82%	3.57E-04	78%
Mouthing	1.41E-08	<1%	1.41E-08	<1%	1.41E-08	<1%	1.25E-08	<1%	6.83E-10	<1%						
Breastfeeding			9.10E-06	1%	1.80E-04	12%										
Total Diet	9.90E-04	51%	3.09E-04	24%	3.09E-04	21%	6.49E-04	40%	3.78E-04	32%	2.15E-04	25%	1.03E-04	18%	1.03E-04	22%
Total	1.94E-03	100%	1.27E-03	100%	1.44E-03	100%	1.63E-03	100%	1.17E-03	100%	8.61E-04	100%	5.84E-04	100%	4.60E-04	100%
<i>Upper-Bound</i>																
Inhalation	2.50E-03	62%	2.50E-03	82%	2.50E-03	53%	2.55E-03	67%	2.07E-03	73%	1.68E-03	79%	1.25E-03	84%	9.32E-04	80%
Mouthing	1.41E-07	<1%	1.41E-07	<1%	1.41E-07	<1%	1.25E-07	<1%	7.51E-08	<1%						
Breastfeeding			2.00E-05	1%	1.70E-03	36%										
Total Diet	1.51E-03	38%	5.32E-04	17%	5.32E-04	11%	1.25E-03	33%	7.77E-04	27%	4.59E-04	21%	2.31E-04	16%	2.31E-04	20%
Total	4.01E-03	100%	3.05E-03	100%	4.73E-03	100%	3.80E-03	100%	2.85E-03	100%	2.14E-03	100%	1.48E-03	100%	1.16E-03	100%

Breastfeeding, which was conservatively assumed here to be additive to dietary intake, had a negligible effect on total intake for children whose mothers were not employed in ethylbenzene related jobs. The most highly exposed population (but only by a very small margin) was the worker's breastfed infant, to whom mother's milk contributed 7% (Urban Smoking) to 14% (Rural/Suburban, Non-Smoking) of total average intake, and 25% to 41% of upper-bound intake. Again the difference in percent contribution between the Rural/Suburban, Non-Smoking scenario and the Urban Smoking scenario was due to the decrease in intake through the inhalation pathway.

As indicated in Section 6.7.3.3, the daily exposure levels associated with toy mouthing were orders of magnitude lower than those associated with other exposure pathways. It was therefore concluded that mouthing of styrenic toys is unlikely to be a significant source of children's exposure to ethylbenzene.

Adult Receptors

Results of estimated intakes for the three adult receptors, At-Home Parent, Office Worker, and Production Worker, are presented in Table 6-59. As with the higher end of the children age groups reported in Table 6-55 through Table 6-58, inhalation was the dominant exposure pathway contributing at least 85% of the total intake. The influence of setting (*e.g.*, urban, smoking and rural/suburban, non-smoking) on magnitude of adult exposure and relative contribution of the inhalation and ingestion pathways was similar to that discussed above for children. However, as expected, the Production Worker's exposure was one to two orders of magnitude greater than those estimated for other adult populations due to the assumption of relatively high workplace exposure.

A comparison of the children's intake and the prospective parent intake for the urban, smoking and rural/suburban, non-smoking exposure setting is presented in Figure 6-9. This figure illustrates the dominant contribution of the inhalation pathway and the decreasing intake with age. The result of the Production Worker, which is a magnitude or more greater than those shown in the figure, were excluded from the presentation.

6.7.5 Chain of Commerce Contribution to Total Ethylbenzene Intake

As discussed in Section 6.7.1.2, the proportion of ethylbenzene in ambient air that is attributable to the ethylbenzene/styrene chain of commerce cannot be precisely quantified, but a conservative estimate is thought to be 1%. Contribution of ethylbenzene attributable to the ethylbenzene/styrene chain of commerce to the population also exposed to ethylbenzene through smoking would be approximately 0.7%. The contribution of migration from food-contact material to the total dietary ethylbenzene concentration was conservatively estimated at 25% (Section 6.7.2.3).

Table 6-59. Total Ethylbenzene Intake: Sum of Inhalation and Ingestion Exposures for Prospective Parent Populations (mg/kg-day)

Pathway	At-Home Parent		Office Worker		Production Worker	
<i>Urban, Smoking</i>						
Central Tendency	Intake	% of Total	Intake	% of Total	Intake	% of Total
Inhalation	1.10E-03	94%	9.67E-04	93%	2.29E-02	100%
Total Diet	7.12E-05	6%	7.12E-05	7%	7.12E-05	0%
Total	1.17E-03	100%	1.04E-03	100%	2.30E-02	100%
Upper Bound						
Inhalation	2.45E-03	94%	2.16E-03	93%	2.23E-01	100%
Total Diet	1.63E-04	6%	1.63E-04	7%	1.63E-04	0%
Total	2.61E-03	100%	2.32E-03	100%	2.23E-01	100%
<i>Urban, Non-Smoking</i>						
Central Tendency						
Inhalation	7.36E-04	91%	6.49E-04	90%	2.27E-02	100%
Diet	7.12E-05	9%	7.12E-05	10%	7.12E-05	0%
Total	8.07E-04	100%	7.20E-04	100%	2.28E-02	100%
Upper Bound						
Inhalation	1.65E-03	91%	1.45E-03	90%	2.22E-01	100%
Diet	1.63E-04	9%	1.63E-04	10%	1.63E-04	0%
Total	1.81E-03	100%	1.61E-03	100%	2.22E-01	100%
<i>Rural/Suburban, Smoking</i>						
Central Tendency						
Inhalation	5.95E-04	89%	5.50E-04	89%	2.26E-02	100%
Diet	7.12E-05	11%	7.12E-05	11%	7.12E-05	0%
Total	6.66E-04	100%	6.21E-04	100%	2.27E-02	100%
Upper Bound						
Inhalation	1.55E-03	90%	1.43E-03	90%	2.22E-01	100%
Diet	1.63E-04	10%	1.63E-04	10%	1.63E-04	0%
Total	1.71E-03	100%	1.59E-03	100%	2.22E-01	100%
<i>Rural/Suburban, Non-Smoking</i>						
Central Tendency						
Inhalation	3.99E-04	85%	3.69E-04	84%	2.24E-02	100%
Diet	7.12E-05	15%	7.12E-05	16%	7.12E-05	0%
Total	4.70E-04	100%	4.40E-04	100%	2.25E-02	100%
Upper Bound						
Inhalation	1.04E-03	86%	9.65E-04	86%	2.22E-01	100%
Diet	1.63E-04	14%	1.63E-04	14%	1.63E-04	0%
Total	1.20E-03	100%	1.13E-03	100%	2.22E-01	100%

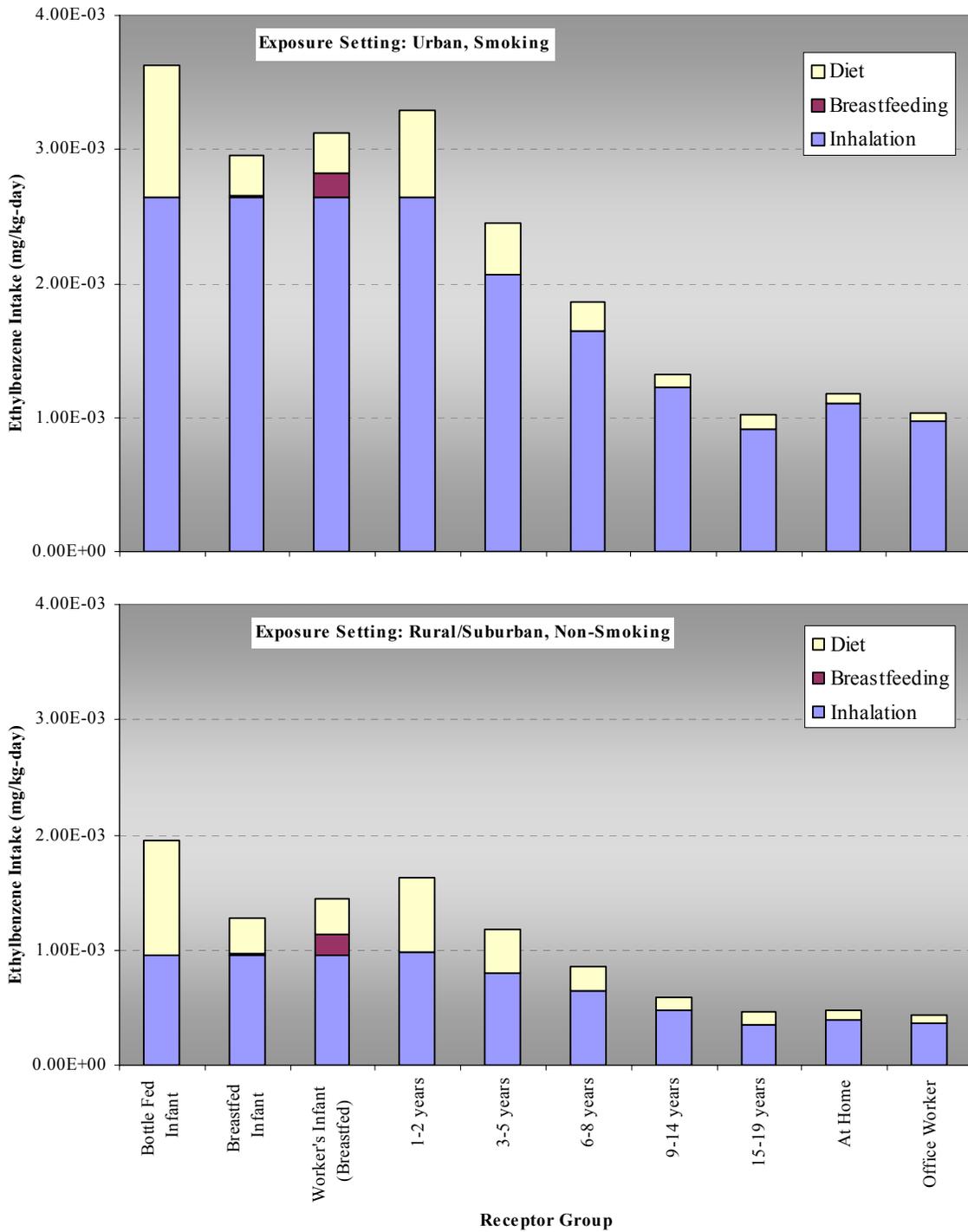


Figure 6-9. Age-Dependence and Relative Contributions of Inhalation and Diet (Central Tendency) to Total Ethylbenzene Intakes in Urban, Smoking vs. Rural/Suburban, Non-Smoking Exposure Settings

Applying these factors to the total intakes via inhalation and dietary ingestion, total intake was generally reduced by at least an order of magnitude for receptors other than the Production Worker, for whom occupational exposure via the inhalation pathway is by far the dominant exposure route, and the Breastfed Worker Child, for whom exposure from the ingestion of breast milk is a major contributor (Table 6-60). The relative contribution of dietary intake was increased because, as described above, only 1% of inhalation intake was considered attributable to the ethylbenzene/styrene chain of commerce while 25% of the dietary intake was considered attributable. Table 6-60 shows the intakes only for the two settings, urban and rural/suburban, because exposure to ethylbenzene via tobacco smoke is not considered applicable to the ethylbenzene/styrene chain of commerce.

Table 6-60. Estimated Ethylbenzene Intakes via Inhalation and Diet Attributable to the Ethylbenzene/Styrene Chain of Commerce (mg/kg-day)

Age Group	Inhalation Intake ^a		Dietary Intake ^b		Total Intake		Chain of Commerce / Total Ethylbenzene ^c	
	Central	Upper	Central	Upper	Central	Upper	Central	Upper
Urban Setting								
<1 year (bottle fed)	1.76E-05	3.94E-05	2.48E-04	3.78E-04	2.65E-04	4.17E-04	9.7%	7.7%
<1 year (breastfed) ^d	1.76E-05	3.94E-05	8.64E-05	1.53E-04	1.04E-04	1.92E-04	5.0%	4.3%
<1 yr (wc breastfed) ^d	1.76E-05	3.94E-05	2.57E-04	1.83E-03	2.75E-04	1.87E-03	12.1%	28.5%
1-2 years	1.79E-05	3.99E-05	1.62E-04	3.13E-04	1.80E-04	3.52E-04	7.4%	6.7%
3-5 years	1.43E-05	3.19E-05	9.45E-05	1.94E-04	1.09E-04	2.26E-04	6.0%	5.7%
6-8 years	1.15E-05	2.57E-05	5.38E-05	1.15E-04	6.53E-05	1.40E-04	4.8%	4.6%
9-14 years	8.60E-06	1.91E-05	2.58E-05	5.78E-05	3.44E-05	7.69E-05	3.6%	3.6%
15-19 years	6.38E-06	1.42E-05	2.58E-05	5.78E-05	3.21E-05	7.20E-05	4.3%	4.4%
At-Home Parent	7.36E-06	1.65E-05	1.78E-05	4.08E-05	2.52E-05	5.73E-05	3.1%	3.2%
Office Worker	6.49E-06	1.45E-05	1.78E-05	4.08E-05	2.43E-05	5.53E-05	3.4%	3.4%
Production Worker	2.27E-02	2.22E-01	1.78E-05	4.08E-05	2.27E-02	2.22E-01	99.6%	100.0%
Rural/Suburban Setting								
<1 year (bottle fed)	9.54E-06	2.50E-05	2.48E-04	3.78E-04	2.57E-04	4.03E-04	13.3%	10.1%
<1 year (breastfed) ^d	9.54E-06	2.50E-05	8.64E-05	1.53E-04	9.59E-05	1.78E-04	7.5%	5.8%
<1 yr (wc breastfed) ^d	9.54E-06	2.50E-05	2.57E-04	1.83E-03	2.67E-04	1.86E-03	18.0%	36.2%
1-2 years	9.78E-06	2.55E-05	1.62E-04	3.13E-04	1.72E-04	3.38E-04	10.5%	8.9%
3-5 years	7.95E-06	2.07E-05	9.45E-05	1.94E-04	1.02E-04	2.15E-04	8.8%	7.5%
6-8 years	6.46E-06	1.68E-05	5.38E-05	1.15E-04	6.02E-05	1.32E-04	7.0%	6.2%
9-14 years	4.81E-06	1.25E-05	2.58E-05	5.78E-05	3.06E-05	7.03E-05	5.2%	4.8%
15-19 years	3.57E-06	9.32E-06	2.58E-05	5.78E-05	2.93E-05	6.71E-05	6.4%	5.8%
At-Home Parent	3.99E-06	1.04E-05	1.78E-05	4.08E-05	2.18E-05	5.12E-05	4.6%	4.3%
Office Worker	3.69E-06	9.65E-06	1.78E-05	4.08E-05	2.15E-05	5.04E-05	4.9%	4.5%
Production Worker	2.24E-02	2.22E-01	1.78E-05	4.08E-05	2.24E-02	2.22E-01	99.6%	100.0%
^a – Values calculated by multiplying the inhalation intake levels for the general population by the estimated contribution from the ethylbenzene/styrene chain of commerce of 1%, except for the Production Worker. ^b – Values calculated by multiplying the total dietary intake levels in the general population by the estimated contribution from the ethylbenzene/styrene chain of commerce of 25%. ^c – Values presented in these columns represent the percent of ethylbenzene intake attributable to the ethylbenzene/styrene chain of commerce to the total intakes estimated in Tables ES-4, ES-6, and ES-7 which are attributable to overall exposure to ethylbenzene. ^d – Total intake includes intake of ethylbenzene due to breastfeeding at a central tendency of 9.1E-06 and upper bound of 2.0E-05 mg/kg-day for the breastfed infant and a central tendency of 1.8E-04 and upper bound of 1.7E-03 mg/kg-day for the worker’s child.								

As indicated in Table 6-60, the percent reduction in total intake from overall exposure to ethylbenzene in the environment to total intake from exposure to ethylbenzene in the ethylbenzene chain of commerce for the Urban Setting ranged from 3.1% for the At-Home Parent to 9.7% for a <1 year old bottle-fed infant based on central tendency values. When based on upper bound values, the ranges were 3.2% to 7.7% for the same receptors. Ranges in the central tendency percent reduction for the Rural/Suburban setting were similar with

values of 4.6% for the At-Home Parent to 13.3% for a <1 year old bottle-fed infant and upper bounds on the same receptors being 4.3% to 10.1%, respectively. For the bottle-fed child, it was assumed that ethylbenzene from the ethylbenzene/styrene chain of commerce contributed 25% of the total. However, infant formula, which is the predominate contributor to total dietary intake may not be packaged in styrenics containers. If that is the case then the reduction in total intake would be considerably greater and the percent contribution less. As shown in Table 6-60, the estimates of percent reduction were less for the Rural/Suburban Setting than the Urban Setting because inhalation exposure was slightly less for the Rural/Suburban Setting than the Urban Setting while dietary exposure did not change.

The reduction in total intake for the production worker, who is assumed to be employed at a facility within the ethylbenzene/styrene chain of commerce, was due to the decrease in exposure via the dietary intake pathway. The major source of exposure for the production worker was by the inhalation pathway, which was assumed to be dominated by workplace exposure.

As indicated in Table 6-60, reduction in the estimated ethylbenzene intake was less for the breastfed worker's child than the non-worker's child, due to dominance of intake through the ingestion of breast milk considered as part of the dietary intake. For the worker's breastfed child, the reduction in the estimated dietary intake was due to the reduction in ethylbenzene content in foods other than breast milk. The concentration in breast milk would not be expected to change significantly because inhalation exposure in the workplace would be the predominant contributor. For the non-worker's breastfed child, the concentration in breast milk was not adjusted and only the change in contribution by way of inhalation and ingestion of other foods by the child was considered. Therefore, the percent reduction for total intake for the non-worker's breastfed child is likely underestimated.

7.0 HAZARD ASSESSMENT

7.1 Introduction

There is an extensive body of literature on the potential health effects from exposure to ethylbenzene. The following text summarizes studies deemed most pertinent to the VCCEP Pilot Program.

All of the toxicology endpoints listed in Tier 1, Tier 2, and Tier 3 of the Pilot Announcement have information available for ethylbenzene. In some cases, multiple studies are available to support an endpoint's toxicity assessment.

The specific studies that correspond to each test listed in each tier of the Pilot Announcement are identified in Table 7-1. Individual studies and other relevant data are described further in the following sections of the hazard assessment, organized by VCCEP category. Additional details concerning individual studies are provided in the OECD SIDS Dossier, SIAR, and International Uniform Chemical Information Database (IUCLID) (Appendix A), and in expanded robust summaries for selected key studies (Appendix O).

Table 7-1. Data Requirements for VCCEP Tiers 1-3 and Key Studies Available for Ethylbenzene

TIER	TEST	RESULTS ^a
1	<p>Acute Toxicity (Oral)</p> <p>(Dermal)</p> <p>(Inhalation)</p>	<p><u>Rat</u> LD₅₀ Oral Toxicity Ethylbenzene has a low order of toxicity following single oral exposure. The Rat Oral LD₅₀ for Ethylbenzene is 5.46 g/kg bwt. Smyth <i>et al.</i> (1962) [RS - 1].</p> <p><u>Rabbit</u> LD₅₀ Dermal Toxicity Ethylbenzene has a low order of toxicity following single dermal exposure. The Rabbit Dermal LD₅₀ for Ethylbenzene is 17.8 mL/kg bwt (15.3 g/kg bwt). Smyth <i>et al.</i> (1962) [RS - 2].</p> <p><u>Rat</u> LC₅₀ Inhalation Toxicity Ethylbenzene has a low order of toxicity following single inhalation exposure. The Rat Inhalation LC₅₀ for Ethylbenzene is 4000 ppm. Smyth <i>et al.</i> (1962) [RS - 3].</p>

TIER	TEST	RESULTS ^a
		1997) [RS – 12]
1	Repeated Dose Toxicity & Reproductive Toxicity (1-Generation) and Developmental Toxicity Screen	Superseded by Tier 2 90-Day Subchronic Toxicity studies, a 2-Generation Reproductive Toxicity study, and Developmental Toxicity studies identified below. NTP (1992a) [RS – 18 and 19] Mellert <i>et al.</i> (2004, 2007) [RS – 20] Stump (2004a) [RS - 21] Andrew <i>et al.</i> (1981), Hardin <i>et al.</i> (1981) [RS – 23 and 24] Saillenfait <i>et al.</i> (2003) [RS - 25]
2	<i>In Vivo</i> Mammalian Bone Marrow Chromosomal Aberrations or Erythrocyte Micronucleus Assay	<i>In Vivo</i> Bone Marrow Erythrocyte Micronucleus Assay Ethylbenzene did not induce increased number of micronucleated polychromatic erythrocytes in mice. NTP (1999) [RS - 13]. Unscheduled DNA Synthesis Ethylbenzene did not induce DNA repair, as measured by Unscheduled DNA Synthesis, in the liver of mice. NTP (1999) [RS - 14].
2	90-Day Subchronic Toxicity in Rodents (Inhalation)	<u>Fischer 344 Rats</u> 90-Day Toxicity Study 90-day inhalation study at 0, 100, 250, 500, 750, 1000 ppm ethylbenzene vapor for 6 hours/day, 5 days/week. No adverse effect on the clinical health or growth of male or female rats except for mild decreases of mean body weight at 1000 ppm. Absolute and/or relative weights of kidney, liver, or lung were increased at 250 ppm and greater. There were no treatment-related histopathologic changes in any tissue. Inflammatory changes in the lung corresponded with lung weight increases but the occurrence and severity of these changes suggested they were probably unrelated to

TIER	TEST	RESULTS ^a
	(Oral)	<p>ethylbenzene. There were no effects on sperm, testicular morphology, or estrous cycle. Since the organ weight changes occurred in the absence of histopathological changes, these findings were not considered adverse and the study NOAEL was considered to be 1000 ppm.</p> <p>Increases in liver and kidney weights but no toxic effects were observed in rats that inhaled \geq 250 ppm ethylbenzene vapor for 13 weeks. NTP (1992a) [RS – 18]</p> <p><u>B6C3F1 Mice</u></p> <p>90-Day Toxicity Study</p> <p>90-day inhalation study at 0, 100, 250, 500, 750, 1000 ppm ethylbenzene vapor for 6 hours/day, 5 days/week. There were no deaths, clinical signs, effects on body weight, or gross pathology findings. There were dose-related higher absolute and relative liver weights in 750 or 1000 ppm males and females and a higher relative kidney weight in 1000 ppm females. There were no treatment-related histopathologic findings in any organs. No differences from control were found in evaluation of sperm or vaginal cytology. Since the organ weights occurred in the absence of any histopathologic change, the study NOAEL was considered to be 1000 ppm.</p> <p>Increases in liver and kidney weights but no toxic effects were observed in mice that inhaled \geq 750 ppm ethylbenzene vapor for 13 weeks. NTP (1992a) [RS – 19]</p> <p><u>Wistar Rats</u></p> <p>90-Day Toxicity Study</p> <p>90-day oral study at 0, 75, 250, 750 mg/kg bwt/day ethylbenzene administered daily by gavage. Survival was unaffected. Clinical signs were post-dose salivation (\geq 250 mg/kg bwt/day) and discolored urine (750 mg/kg bwt/day), a finding that was not replicated in urinalysis at study termination. Body weight and feed efficiency for 750 mg/kg bwt/day males was lower than controls. Water consumption was increased at \geq 250 mg/kg bwt/day and attributed to local digestive</p>

TIER	TEST	RESULTS ^a
		<p>tract irritation. Landing foot splay was decreased in 750 mg/kg bwt/day males which may have been related to decrease in body weights. Motor activity was increased in 750 mg/kg bwt/day females but by an atypical pattern suggesting an incidental finding. Hematology changes of increased mean corpuscular volume at ≥ 250 mg/kg bwt/day and decreased platelet counts in 750 mg/kg bwt/day females indicated a mild regenerative anemia. Prothrombin times were shorter in ≥ 250 mg/kg bwt/day animals of both sexes. A number of clinical chemistry changes were noted at ≥ 250 mg/kg bwt/day that could have been due to liver enzyme induction or secondary to the feed and water effects. Compound related pathology changes occurred in the liver of both sexes and in the male kidney at ≥ 250 mg/kg bwt/day. The liver effects may have been due to a metabolic adaptive response and the male kidney effects may have been due to α-2u-globulin increases. The study NOAEL was concluded to be 75 mg/kg bwt/day.</p> <p>Changes in hematology, indicative of a mild regenerative anemia, and clinical chemistry parameters, indicative of hepatic microsomal enzyme induction, decreases in prothrombin time, mild alimentary effects and kidney (males only) and liver pathology were observed in rats that received gavage doses of ≥ 250 mg/kg bwt/day ethylbenzene for 90 days. Mellert <i>et al.</i> (2004, 2007) [RS – 20]</p>
2	<p>Reproduction and Fertility Effects</p> <p>(Inhalation)</p>	<p><u><i>Sprague-Dawley Rats</i></u></p> <p>Two-Generation Reproductive Toxicity Study</p> <p>Two-generation inhalation study at 0, 25, 100, 500 ppm ethylbenzene vapor for 6 hours/day, 7 days/week, with inhalation suspension of dams on gestation day 20 through lactation day 4. On lactation days 1 through 4, dams received daily oral gavage doses of 0, 26, 90, or 342 mg/kg bwt/day administered as 1/3 divided doses three times per day at approximately 2 hour intervals. There were no test article-related deaths or clinical observations. Mild effects on body weight gain were observed in parental animals at 500 ppm. Increases in absolute and relative liver and kidney weights were present in 500 ppm F₀ and F₁ males. No gross or</p>

TIER	TEST	RESULTS ^a
		<p>microscopic pathology findings were related to test article exposure. There were no test article-related effects on reproductive performance. F₁ and F₂ pup sex ratios, live litter sizes, number of dead pups, viability indices, pup body weights, the general physical condition of the pups, and pre- and post-weaning developmental landmarks were not adversely affected by ethylbenzene exposure.</p> <p>Parental Toxicity NOAEL – 500 ppm or 500 ppm/342 mg/kg bwt/day, NOEL – 100 ppm or 100 ppm/90 mg/kg bwt/day</p> <p>Reproductive Toxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt/day</p> <p>Developmental Toxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt/day</p> <p>No parental, neonatal, or reproductive toxicity was observed following inhalation exposure of rats to up to 500 ppm or 500 ppm/342 mg/kg bwt/day ethylbenzene over two generations. Stump (2004a), Faber <i>et al.</i> (2006) [RS - 22]</p>
2	<p>Prenatal Developmental Toxicity (two species)</p> <p>(Inhalation)</p>	<p><u>Wistar Rats</u></p> <p>Inhalation study at 0, 100, or 1000 ppm ethylbenzene, 7 hours/day, 3 weeks before fertilization and from days 1 to 19 of gestation. There were no effects on maternal survival and body weight and no evidence of histological damage in any of the dams' organs. Both absolute and relative liver, kidney, and spleen weights were significantly increased in the dams at 1000 ppm. There was no evidence of fetal toxicity or increases in major malformations. At 1000 ppm, there was a significant increase in the incidence of skeletal variations (supernumerary ribs). The skeletal variants in this study were considered marginally adverse.</p> <p>Maternal Toxicity NOAEL – 1000 ppm, NOEL – 100 ppm</p> <p>Developmental Toxicity NOAEL – 100 ppm</p> <p>Ethylbenzene is not a teratogen in rats. Ethylbenzene produced a mild increase in fetal effects (increased incidence in skeletal variations) in concert with mild maternal increases in organ</p>

TIER	TEST	RESULTS ^a
		<p>weights in rats at 1000 ppm. Andrew <i>et al.</i> (1981), Hardin <i>et al.</i> (1981) [RS - 23]</p> <p><u>Sprague-Dawley Rats</u></p> <p>Inhalation study at 0, 100, 500, 1000, or 2000 ppm Ethylbenzene, 6 hours/day, during days 6 to 20 of gestation. There were no effects on maternal survival. Clinical signs of toxicity (ataxia, decreased motor activity) were present at 2000 ppm. Maternal body weight, body weight gain, and feed consumption were significantly reduced at ≥ 1000 ppm. No evidence of teratogenic effects was found at any exposure level. Fetal body weights were significantly decreased at ≥ 1000 ppm. A significant increase in the mean percentages of fetuses per litter with skeletal variations was noted at 2000 ppm.</p> <p>Maternal Toxicity NOAEL - 500 ppm</p> <p>Developmental Toxicity NOAEL - 500 ppm</p> <p>Ethylbenzene is not a teratogen in rats. Ethylbenzene produced a mild increase in fetal effects (decreased body weights and increased incidence in skeletal variations) in concert with maternal effects (clinical signs and body weight decreases) in rats at ≥ 1000 ppm. Saillenfait <i>et al.</i> (2003) [RS - 25]</p> <p><u>New Zealand White Rabbits</u></p> <p>Inhalation study at 0, 100, or 1000 ppm ethylbenzene, 7 hours/day, Days 1 to 24 of gestation. There were no effects on maternal survival and body weight and no evidence of histologic damage in any of the does' organs. Relative liver weights were slightly increased in 1000 ppm does but absent any accompanying histopathological changes, this finding was not considered biologically relevant. There were no treatment-related developmental toxic effects. There was a slight statistically significant decrease in the number of live fetuses/litter in the 1000 ppm group. The finding was considered equivocal due to no corresponding increases in other parameters (implantations, resorptions, dead fetuses, etc.). There were no significant changes in the incidence of</p>

TIER	TEST	RESULTS ^a
		<p>variations or malformations in the rabbit pups. Maternal Toxicity NOAEL – 1000 ppm, NOEL – 100 ppm Developmental Toxicity NOAEL – 1000 ppm Ethylbenzene is not a teratogen in rabbits. Ethylbenzene did not elicit maternal or developmental toxicity in rabbits at 1000 ppm. Andrew <i>et al.</i> (1981), Hardin <i>et al.</i> (1981) [RS – 24]</p>
2	Immunotoxicity	<p><u>Sprague-Dawley Rats</u> Splenic Antibody Formation Study 28-day inhalation study at 0, 25, 100, 500 ppm ethylbenzene vapor for 6 hours/day for 28 consecutive days. All animals received a single intravenous immunization injection via a lateral tail vein of sheep red blood cells approximately 4 days prior to the scheduled necropsy. There were no treatment-related effects on survival, clinical signs, body weight, or feed consumption. Hematology parameters were not affected by ethylbenzene exposure. Liver and kidney weights relative to final body weights were increased in the 500 ppm group. There were no treatment-related effects of ethylbenzene on IgM Antibody Forming Cell Response. The positive control article, cyclophosphamide, performed as expected, exhibiting a decrease in spleen and thymus weights and a decrease in spleen cell numbers and IgM Antibody Forming Cell response. The study NOAEL was concluded to be 500 ppm. Ethylbenzene at up to 500 ppm vapor concentration did not adversely affect the functional ability of the humoral component of the immune system of rats as measured by splenic IgM antibody forming cell response to the T-dependent antigen, sheep erythrocytes. Stump (2004b) [RS - 26]</p>
2	Metabolism and Pharmacokinetics	<p>Ethylbenzene is well absorbed from the skin, lungs and gastrointestinal tract, rapidly distributed in the body, metabolized primarily via hydroxylation of the two carbons of the side-chain and then further oxidized to a range of metabolites that are excreted principally in the urine. Differences are apparent</p>

TIER	TEST	RESULTS ^a
		<p>between animal species in the urinary metabolite profiles and overall clearance of ethylbenzene. [Database Summary]</p>
<p>3</p>	<p>Carcinogenicity or Combined Chronic Toxicity / Carcinogenicity.</p> <p>(Inhalation)</p>	<p><u>Fischer 344 Rats</u></p> <p>Combined Chronic Toxicity and Carcinogenicity Study</p> <p>2-year inhalation study at 0, 75, 250, 750 ppm ethylbenzene vapor for 6 hours/day, 5 days/week. Survival was significantly reduced in 750 ppm males. Mean body weights of 250 and 750 ppm males were generally lower throughout the study; exposed females had body weights generally lower in the second year of the study. Treatment-related organ pathology was present in the kidney and testes. In a standard single section of each kidney, there was a significant increase in renal tubule tumors (adenomas and adenomas and carcinomas combined) in 750 ppm males, but not females. Step-sectioning of the kidneys found additional adenomas confirming an increase in males and revealing an increase in females. Focal renal tubule hyperplasia was also increased in incidence in the 750 ppm rat kidneys. The severity of nephropathy was significantly increased in 750 ppm males and all exposed female groups. In the testis, the incidence of interstitial cell adenoma in 750 ppm males was significantly increased.</p> <p>A decrease in survival and body weight and an increase in kidney pathology (renal tubule hyperplasia and nephropathy) were observed in rats that inhaled ≥ 75 ppm ethylbenzene for 2 years. At 750 ppm ethylbenzene, male rats exhibited increased incidences of kidney (renal tubule neoplasms) and testes tumors (testicular adenoma) and female rats also showed kidney tumors (renal tubule adenomas), but in a lower incidence and only detected after extended evaluation. NTP (1999) [RS - 27]</p> <p><u>B6C3F1 Mice</u></p> <p>Combined Chronic Toxicity and Carcinogenicity Study</p> <p>2-year inhalation study at 0, 75, 250, 750 ppm ethylbenzene vapor for 6 hours/day, 5 days/week. Survival was unaffected by treatment and consistent body weight effects were not observed. Treatment-</p>

TIER	TEST	RESULTS ^a
		<p>related organ pathology was present in the lung, liver, thyroid, and pituitary. In the lung, the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma were significantly greater in 750 ppm males compared to chamber controls but were within the historical control range. The incidence of alveolar epithelial metaplasia was significantly greater in 750 ppm males. In the liver, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma were significantly greater in 750 ppm females than chamber controls but were within the historical control range. 750 ppm Females also had an increased incidence of eosinophilic foci, a lesion judged to be a precursor of hepatocellular adenoma. There were also increased incidences of hepatocyte syncytial alteration, hypertrophy, and necrosis in the livers of 750 ppm males and increased syncytial alteration of hepatocytes in 250 ppm males. In the thyroid, 750 ppm males and females had significantly greater incidences of thyroid follicular cell hyperplasia. In the pituitary, 250 and 750 ppm females exhibited significantly increased incidences of hyperplasia of the pituitary gland pars distalis.</p> <p>Liver, lung, thyroid, and pituitary pathology was observed in mice that inhaled ≥ 250 ppm ethylbenzene for 2 years. At 750 ppm ethylbenzene, male mice exhibited lung tumors (alveolar/bronchiolar neoplasms) and female mice exhibited liver tumors (hepatocellular neoplasms) at incidences greater than chamber controls but within the laboratory historical control incidence range. NTP (1999) [RS - 28]</p>
3	<p>Neurotoxicity Screening Battery (Inhalation)</p>	<p><u><i>CFW Mouse</i></u> Acute Neurotoxicity</p> <p>Ethylbenzene produced neurobehavioral changes in mice during and shortly after receiving 20-minute exposures to ≥ 2000 ppm. Tegeris and Baltser (1994) [RS - 29]</p> <p><u><i>Sprague-Dawley Rat</i></u> Subchronic Ototoxicity</p> <p>90-day inhalation study at 0, 200, 400, 600, 800 ppm</p>

TIER	TEST	RESULTS ^a
	(Gavage)	<p>ethylbenzene vapor for 6 hours/day, 6 days/week with an 8 week post exposure recovery period. There were 2 deaths and an animal removed from the study in the 800 ppm group. No significant differences were observed in weight gain between the controls and the groups exposed to ethylbenzene. Concentrations of 400 ppm and greater produced significantly higher audiometric thresholds that did not recover 8 weeks after exposure ceased. Following the 8 week recovery period, Outer Hair Cell losses were present with increasing severity (4% to nearly 100%, respectively) in the rats that received 200 to 800 ppm ethylbenzene.</p> <p>Ethylbenzene produced moderate to severe ototoxicity in young adult rats exposed for 4 to 13 weeks to concentrations ranging from 200 to 800 ppm. Gagnaire <i>et al.</i> (2007) [RS – 34]</p> <p><u>Sprague-Dawley Rat</u></p> <p>Subchronic Neurotoxicity</p> <p>90 day oral study at 0, 50, 250, and 500 mg/kg bwt/day ethylbenzene administered by gavage daily as ½ divided doses 2 times per day approximately 3 hours apart. Behavioral tests (motor activity and functional observational battery) were conducted prior to initiation of dosing and during the 4th, 8th and 13th week of exposure. Immediately after exposure, rats were perfused <i>in situ</i> and kidneys, livers, and nervous system tissues were evaluated in high dose and control animals. No adverse exposure-related effects on mortality, findings in functional observational battery assessments, motor activity, or histopathology of the nervous system, kidneys and livers were noted. Significant changes in body weight gains and/or feed consumption values occurred at several weekly intervals in the groups given 250 or 500 mg/kg bwt/day but values for the entire dosage period were generally comparable to the controls. At 500 mg/kg bwt/day, there were slight increases in the numbers of male and female rats observed with slight to moderate excess salivation and marginal increases in urine-stained abdominal fur. The majority of observations of excess salivation were observed around the time that the daily doses were administered. At 500 mg/kg bwt/day, the</p>

TIER	TEST	RESULTS ^a
		<p>following few statistically significant findings on the FOB were considered incidental: (a) increased incidence in female rats having normal levels of urination in the open field (week 4); (b) an increased incidence in female rats having a startle reaction to acoustic stimuli (week 8); and (c) decreased incidence in male rats having a startle reaction to acoustic stimuli (week 13). These observations were not considered adverse because they occurred with similar incidence to that observed in groups during pre-test or in controls during exposure in this study. There was also a statistically significant trend in pattern of intersession activity in the 250 and 500 mg/kg bwt/day dose group during the 4th week. This was considered incidental because there were no consistent dose-related pattern in the average intersession data (e.g. values for the female group given 250 mg/kg bwt/day generally exceeded those at 500 mg/kg bwt/day; values for the group given 50 mg/kg bwt/day were lower than controls), and there were no statistically significant differences after longer exposure durations. Absolute organ weights for the liver and paired kidneys were increased for both the male and female rats at 250 and 500 mg/kg bwt/day, and relative weights of these organs to terminal body weights were increased in both male and female rats at the 250 and 500 mg/kg bwt/day dosage groups. Absolute brain weights in the male and female rats were unaffected at 500 mg/kg bwt/day. A statistically significant increase in the ratio of brain weight to terminal body weight is attributed to the slight decrease in terminal body weight that occurred in the 500 mg/kg bwt/day dosage group. There were no treatment-related histopathology findings of the liver, kidney or nervous system tissues.</p> <p>Systemic Toxicity NOEL - 50 mg/kg bwt/day</p> <p>Adult Neurotoxicity NOEL - 500 mg/kg bwt/day</p> <p>Ethylbenzene did not cause neurotoxic effects in young adult rats following repeated daily exposure to doses up to 500 mg/kg bwt/day. Barnett (2006); [RS-33]</p>

TIER	TEST	RESULTS ^a
3	Developmental Neurotoxicity	<p><u>Sprague-Dawley Rats</u></p> <p>Developmental Neurotoxicity Study (Component of Two Generation Reproductive Toxicity Study)</p> <p>First generation (F₁) dams of a two generation inhalation study received 0, 25, 100, 500 ppm ethylbenzene vapor for 6 hours/day, 7 days/week, with inhalation suspension on gestation day 20 through lactation day (LD) 4. On LD 1 through 4, dams received daily oral gavage doses of 0, 26, 90, or 342 mg/kg bwt/day administered as 1/3 divided doses 3 times per day at approximately 2 hour intervals. Pups (F₂) received no direct exposure; offspring potentially exposed <i>in utero</i> and through nursing during lactation. No adverse exposure-related effects on survival, clinical observations, findings in functional observational battery assessments, or in macroscopic findings were noted at any exposure level in the F₁ generation dams or in the F₂ offspring. F₂ offspring body weight data, pre-weaning and post-weaning developmental landmarks, auditory startle habituation, motor activity, Biel water maze learning and memory assessments, organ weights, microscopic pathology and brain morphometry parameters were unaffected by ethylbenzene exposure. On PND 60, F₂ males from all ethylbenzene exposure groups exhibited lower mean peak startle amplitudes, and on PND 61, F₂ females from the low-exposure group were significantly more active than the concurrent controls. These apparent behavioral shifts did not occur in a dose-responsive manner, fell within the historical control ranges in this laboratory, and were not attributed to parental ethylbenzene exposure.</p> <p>Parental Toxicity NOAEL – 500 ppm or 500 ppm/342 mg/kg bwt/day, NOEL – 100 ppm or 100 ppm/90 mg/kg bwt/day Developmental Neurotoxicity NOAEL - 500 ppm or 500 ppm/342 mg/kg bwt</p> <p>Ethylbenzene at an exposure level of 500 ppm/342 mg/kg bwt/day did not adversely affect neurodevelopment in rats. Stump (2004a), Faber <i>et al.</i> (2007)[RS - 35]</p>

^a Robust Summaries (RS - 1 through 35) for these key studies are provided in Appendix O .

7.2 Acute Toxicity (Tier 1)

The acute toxicity of ethylbenzene has been extensively examined. Animal studies have focused primarily on lethality, narcosis, and sensory irritation, and have been conducted in multiple species (rats, mice, guinea pigs, rabbits) and by multiple routes of administration (oral, inhalation, dermal).

Estimates of the acute oral LD₅₀ in rats range from approximately 3.5 to 5.46 g/kg bwt (Wolf *et al.*, 1956; Smyth *et al.*, 1962) and, for acute dermal LD₅₀, values for rabbits range from greater than 15.3 g/kg bwt (Smyth *et al.*, 1962) to greater than 20 g/kg bwt (Harton and Rawl, 1986).

A number of laboratory animal studies have assessed the acute inhalation toxicity of ethylbenzene. Overall high vapor concentrations were required to produce mortality with lower concentrations producing mucous membrane irritation and a variety of central nervous system effects. The study with experimental design most comparable to a modern guideline acute inhalation lethality study was conducted by Smyth *et al.* (1962). This study exposed groups of rats to various vapor concentrations of ethylbenzene (only nominal concentrations provided) for 4 hours and determined mortality over the course of 14 days. A concentration of 4000 ppm (17360 mg/m³) was found to be lethal to 3 of 6 exposed rats that can be taken as the LC₅₀ value for this study.

A couple of acute inhalation toxicity studies have been conducted for ethylbenzene to assess neurological effects. Molnar *et al.* (1986) exposed rats to ethylbenzene vapor at concentrations ranging from approximately 100 to 3000 ppm (434 to 13020 mg/m³) for up to 4 hours and measured group motility during the exposure. Rats that were exposed to 400 to 1500 ppm (1736 to 6510 mg/m³) ethylbenzene exhibited moderate activation in motor activity and exposure to 2180 ppm (9461 mg/m³) ethylbenzene caused minimal narcotic effects. A thorough Functional Observational Battery protocol was performed by Tegeris and Baltser (1994) on mice during and shortly after a 20 min exposure to 2000, 4000 or 8000 ppm ethylbenzene (8680, 17360 or 34720 mg/m³). A number of neurobehavioral changes were observed in these mice both during and after exposure to ≥ 2000 ppm ethylbenzene.

Other studies that reported lethal vapor concentrations for ethylbenzene are Ivanov (1962) that noted an LC₅₀ of ca. 13367 ppm (58012 mg/m³) in rats exposed for 2 hours, Gerarde (1960) that reported a lethal concentration of 10000 ppm (43400 mg/m³) in mice (no exposure period given), and Yant *et al.* (1930) that reported death in guinea pigs exposed for a few minutes to 10000 ppm. The Yant *et al.* (1930) study reported clinical neurological and irritancy signs and gross pathology in guinea pigs exposed to 1000 to 10000 ppm (4340 to 43400 mg/m³) ethylbenzene vapor. At 1000 ppm eye irritation was noted and at 2000 (8680 mg/m³) to 10000 ppm signs included eye and nose irritation, dizziness, ataxia, tremor, respiratory disorders and narcosis. Gross pathology (presumably in the deceased animals) consisted of congestion in the brain and pulmonary edema.

A couple of studies have been conducted that assessed the sensory irritation effects of ethylbenzene. Nielsen and Alarie (1982) reported an RD₅₀ (concentration that produces a 50% decrease in respiratory rate) of 4060 ppm (17620 mg/m³) in ethylbenzene-exposed mice. De Ceaurriz *et al.* (1981) found an RD₅₀ of 1432 ppm (6215 mg/m³) in mice for a 5

minute exposure. As these studies indirectly evaluated sensory irritation via respiratory depression, their interpretation is possibly conflicted by the acute central nervous system effects that are also known to be produced by ethylbenzene.

Acute exposures to ethylbenzene can cause irritation to skin, eyes and mucous membranes. Inhalation of ethylbenzene vapor at a concentration of 1000 ppm (4300 mg/m³) for 3 minutes caused slight nasal irritation in guinea pigs and an 8 minute exposure caused eye irritation as well. At 2000 ppm (8680 mg/m³), a 1 minute exposure produced both effects and at a moderate level (Yant *et al.*, 1930).

Two drops of undiluted ethylbenzene liquid placed in the eyes of rabbits resulted in slight conjunctival irritation but no effects to the cornea (Wolf *et al.*, 1956). A slight conjunctival irritation with some reversible corneal injury was reported in rabbits in a study by Smyth *et al.* (1962).

Undiluted ethylbenzene liquid has been shown to produce moderate irritation when applied to the uncovered skin of rabbits (Smyth *et al.*, 1962). The application of undiluted ethylbenzene liquid to the ear and shaved abdomen of rabbits up to 10 times during a 4-week period resulted in moderate irritation. There was erythema and edema evident with superficial necrosis and exfoliation of large patches of skin (Wolf *et al.*, 1956).

No experimental animal skin sensitization studies have been conducted for ethylbenzene.

In summary, ethylbenzene's acute toxicity has been well characterized in multiple animal species and by multiple routes of exposure and the results demonstrate that ethylbenzene overall has low acute toxicity. Accordingly, no further testing of acute toxicity of ethylbenzene is warranted.

7.3 Metabolism and Pharmacokinetics (Tier 2)

Numerous studies have evaluated ethylbenzene's metabolism and kinetics in mammalian systems. Studies examined neat ethylbenzene and also ethylbenzene as a component of mixed xylenes; the latter information, however, has not been included in this overview. Below are summaries of the reported ethylbenzene metabolism and pharmacokinetic studies discussed with regards to ethylbenzene's absorption, distribution, excretion, metabolism, and toxicokinetics.

7.3.1. Absorption

Ethylbenzene is rapidly absorbed following inhalation or ingestion. Absorption by the skin is also rapid if volatilization is impeded.

In Wistar rats exposed to 1000 mg/m³ (230 ppm) ¹⁴C-(ring)-ethylbenzene for 6 hours, ethylbenzene uptake was determined to be 44% (Chin *et al.*, 1980). In humans exposed to 23 to 85 ppm (100 to 369 mg/m³) for 8 hours, it was reported that "64% was retained in the respiratory tract" (Bardodej and Bardodejova, 1970). In 12 human volunteers, the uptake of ethylbenzene was highly correlated with the amount of body fat (Engström and Bjurström, 1978). When volunteers were exposed for 2 hours to 435 or 870 mg/m³ (100 or 200 ppm) of "industrial xylene" (containing 40% ethylbenzene and 60% xylenes), about 65% was taken

up by the lungs. If the workload was increased during exposure, the retention dropped to 50% (Åstrand *et al.*, 1978). Using 6 human volunteers, Gromiec and Piotrowski (1984) calculated the retention of ethylbenzene in lung to be $49 \pm 5\%$.

There are no studies of absorption of ethylbenzene in humans following oral exposure. An earlier study in rabbits reported recovery of 72 to 92% of an oral dose of 593 mg/kg bwt in urine 24 hours following dosing (El Mastri *et al.*, 1956).

Several studies have examined the absorption of ethylbenzene through skin. The *in vitro* penetration rate using rat skin was $0.993 \text{ nmole/cm}^2/\text{minute}$ ($0.1 \text{ }\mu\text{g/cm}^2/\text{minute}$) (Tsurata, 1982). The rate was 5-fold less than styrene and 8-fold less than toluene. Morgan *et al.* (1991) measured the penetration of a number of organic solvents through the skin of rats *in vivo* by assaying blood levels during application to the skin for 24 hours. The peak level of $5.6 \text{ }\mu\text{g ethylbenzene/mL blood}$ occurred at 1 hour. The blood level was very similar to that of styrene ($5.3 \text{ }\mu\text{g/mL}$) and somewhat less than that of toluene ($9.5 \text{ }\mu\text{g/mL}$). The absorption rate of ethylbenzene was determined in hairless (HRS/J) mice by applying a large excess in a closed exposure chamber glued to the back for 4 hours (Susten *et al.*, 1990). For ethylbenzene, the absorption rate was determined to be $37 \pm 31 \text{ }\mu\text{g/cm}^2/\text{minute}$. Total absorption (sum of radioactivity found in carcass, excreta, skin application site and expired breath) was 3.4% of the nominal dose.

Dutkiewicz and Tyras (1967) measured the absorption of ethylbenzene in humans as the difference between the amount applied under a watch glass tightly fixed to forearm skin (174 mg in area of 17.3 cm^2) and the amount extracted into ethanol after 10 or 15 minutes. They reported absorption of 64 to 96 mg after 10 minutes in four subjects and 104 to 130 mg after 15 minutes in 3 subjects; they calculated rates of 22-33 $\text{mg/cm}^2/\text{hour}$ ($365\text{-}550 \text{ }\mu\text{g/cm}^2/\text{minute}$) for neat ethylbenzene from these data. They also calculated the rate of absorption of ethylbenzene from aqueous solutions of 110 to 162 ppm as the difference in concentration before and after immersion of a whole hand in the solution for 1 hour in a one-liter beaker. Loss of ethylbenzene by volatilization was minimized by placing the beaker in a polyethylene bag that was tightly fixed to the forearm above the wrist. Absorption was estimated to be 109 to 120 $\mu\text{g/cm}^2/\text{hour}$ from aqueous solutions of approximately 112 mg/L in a total of 7 trials using 5 people and 201-229 $\mu\text{g/cm}^2/\text{hour}$ from aqueous solutions of approximately 156 mg/L in a total of 7 trials using 4 people. To further complicate the absorption picture, the authors also exposed 5 of these subjects to ethylbenzene in water (concentration not reported) by submerging both hands for 2 hours. "Direct absorption" was measured as the difference in ethylbenzene concentration in the water before and after exposure. They estimated absorption of rates of 174 to 199 $\mu\text{g/cm}^2/\text{hour}$, but reported that only 3.5 to 6% of the "absorbed" ethylbenzene was excreted as mandelic acid within 24 hours. These data are not consistent among experimental conditions. A slight increase in concentration in water (from 112 to 156 mg/L) is reported to double the absorption rate and application of neat ethylbenzene increased absorption 100-fold.

Absorption of ethylbenzene vapor through the skin in humans appears to be minimal (Gromiec and Piotrowski, 1984).

7.3.2. Distribution

Absorbed ethylbenzene is rapidly distributed within the body. Rats exposed by inhalation to 1000 mg/m³ (230 ppm) ¹⁴C-ring-labeled ethylbenzene for 6 hours and held in closed metabolism cages for 72 hours, showed very low levels of ethylbenzene in representative tissues. Total tissue radioactivity was less than 0.2% of the inhaled dose. Liver contained 0.014% of the absorbed dose, while fat contained 0.007%. Lung content was 0.006% and the gastrointestinal tract content was 0.008%. All other tissues contained less than 0.003% (Chin *et al.*, 1980). In rats exposed to 0, 50, 300, or 600 ppm (0, 217, 1302 or 2604 mg/m³) ethylbenzene, 6 hours/day, 5 days/week for up to 16 weeks, there was a dose-related increase in the concentration of ethylbenzene in perirenal fat, although it was not proportional to dose (Engström *et al.*, 1985).

Wag/Rij rats and albino guinea pigs were exposed to 500 ppm (2179 mg/m³) ethylbenzene vapor, 8 hours/day for 3 days. At the end of the first day's exposure, the blood concentration of ethylbenzene in rats and guinea pigs were 23.2 and 2.8 ng/mL, respectively. After 3 days of exposure the concentration difference was 4.3-fold between rats and guinea pigs, although the ethylbenzene concentration in both species had decreased (Cappaert, 2000).

In the mouse skin absorption study by Susten *et al.* (1990) described above, the percentages of absorbed dose following dermal application were 15.5% in carcass, 4.5% in skin at application site, 14.3% in expired breath, and 65.5% in excreta.

7.3.3. Excretion

The principal route of excretion from both oral and inhalation exposure to ethylbenzene is through urine. In rats, about 83% of the radioactivity of ethylbenzene inhaled during 6 hours of exposure at 1000 mg/m³ (230 ppm) was excreted in the urine during the next 72 hours. About 8% was exhaled, 0.7% was excreted in the feces, 0.03% in exhaled CO₂, 8.2% in expired gases, 0.2% remained in the tissues, and 8.3% could not be accounted for (Chin *et al.*, 1980).

In human volunteers exposed to "industrial xylene" (containing 40% ethylbenzene and 60% xylenes) for two hours, about 4% of the ethylbenzene plus xylenes that was taken up by the lungs was exhaled unchanged during the next 19 hours (Åstrand *et al.*, 1978). Elimination from fat was slow; there was little change in the fat concentration of ethylbenzene plus xylenes between the 4-hours post-exposure period and the 22-hour post-exposure period (Engström and Bjurström, 1978). In human volunteers exposed for 40 minutes to a variety of consumer products including air fresheners, paint solvents, dry-cleaning solvents, moth proofing agents, etc., the half-life of ethylbenzene elimination was 5.5 hours based on declines of exhaled breath ethylbenzene concentration of volunteers in a "clean-air chamber" (Pellizzari *et al.*, 1992).

7.3.4. Metabolism

Two very different metabolic pathways for ethylbenzene have been cited in the literature through the α - or ω -oxidation of the side chain by cytochrome P-450 isozymes to 1- and 2-phenylethanol, respectively. The initial step of ω -oxidation of ethylbenzene to 2-phenylethanol leads to phenylacetic acid, which is conjugated with glycine to form phenacetic acid (Kiese and Lenk, 1974). The major pathway, however, is the α -oxidation of ethylbenzene to 1-phenylethanol, which has been shown to be under stereochemical control. 1-Phenylethanol excreted in the urine of rats dosed with ethylbenzene produced about 90% R(+)- and 10% S(-) 1-phenylethanol (McMahon and Sullivan, 1966). *In vitro* liver microsomal metabolism produced about 80% R(+)- and 20% S(-) 1-phenylethanol (McMahon and Sullivan, 1966). Phenobarbital pretreatment substantially diminished the stereospecificity of the microsomal hydroxylation of ethylbenzene (McMahon and Sullivan, 1966; McMahon and Sullivan, 1968). The subsequent intermediates are acetophenone, ω -hydroxyacetophenone, phenyl-glyoxal, phenylglyoxylic acid, and finally hippuric acid.

The pattern of the urinary metabolite excretion seems to vary with different mammalian species. In humans, ethylbenzene is mainly excreted in the urine as mandelic acid and phenylglyoxylic acids (Bardodej and Bardodejova, 1970; Åstrand *et al.*, 1978; Engström *et al.*, 1984; Gromiec and Piotrowski, 1984; Kawai *et al.*, 1992; Knecht *et al.*, 2000). The elimination of mandelic acid has been found to be biphasic, with half-lives of 3.1 and 24.5 hours (Gromiec and Piotrowski, 1984). In rats and rabbits, hippuric acid and phenacetic acid are the main metabolites of ethylbenzene (Kiese and Lenk, 1974; Engström, 1984).

Direct ring oxidation of ethylbenzene occurs to a limited extent. In humans, the combined share of 4-ethylphenol, *m*- and *p*-hydroxyacetophenones accounted for approximately 4% of the total amount of metabolites excreted (Engström *et al.*, 1984). In rats, the share of these compounds was even less (Engström, 1984). Angerer and Lehnert (1979) reported that between 1.0 and 1.4% of ethylbenzene (exposure at 34 to 41 ppm)(147 to 178 mg/m³) was metabolized in humans to 2-ethylethanol. *In vitro* experiments have demonstrated that both 2- and 4-ethylphenol can readily be formed from ethylbenzene, if the reaction is fortified with rat liver microsomes (Kaubisch *et al.*, 1972).

In vivo conversion of ethylbenzene to mandelic acid is stereoselective, and the R-enantiomer is mainly excreted in the urine (Sullivan *et al.*, 1976; Drummond *et al.*, 1989). Rats only excrete the R-enantiomer of mandelic acid in the urine when dosed with ethylbenzene (McMahon and Sullivan, 1968; Drummond *et al.*, 1989). When 2 human volunteers were exposed by inhalation to 430 mg/m³ (100 ppm) ethylbenzene for four hours, only the mandelic acid was excreted as the R-enantiomer (Drummond *et al.*, 1989). In urine taken from workers at the end of a workshift in a plant that made aromatic solvents containing ethylbenzene (ethylbenzene exposures were 1.5 to 33 ppm (6.5 to 143 mg/m³); the workers were not exposed to styrene), the ratio of R- to S-mandelic acid was 19:1 and was independent of airborne ethylbenzene concentration (Korn *et al.*, 1992).

In rats exposed to 0, 50, 300, or 600 ppm (0, 217, 1302 or 2604 mg/m³) ethylbenzene, 6 hours/day, 5 days/week for up to 16 weeks, a significant dose-related decrease of phenylglyoxylic acid and hippuric acid plus benzoic acid was found in the urine. A

corresponding increase of 1-phenylethanol and ω -hydroxyacetophenone excretion was noted. The total amount of metabolites in the urine, collected during 24 hours after onset of exposure remained constant at each exposure level throughout the study (Engström *et al.*, 1985).

Significant lung metabolism of ethylbenzene was demonstrated in rabbits in an *in vitro* study conducted by Sato and Nakajima (1987). The rate of metabolism was 453 nmol/g/10 minutes in liver and 680 nmol/g/10 minutes in lungs; thus indicating that the lung may significantly contribute to the body clearance of ethylbenzene in rabbits.

Exposure of rats to very high doses of ethylbenzene either induce or inhibits a number of cytochrome P-450 isozymes over different time courses. Cytochrome P-450 2E1 protein levels increased about 2-fold after a single intraperitoneal injection of 1060 mg/kg bwt ethylbenzene, which was followed by a slight increase in cytochrome P-450 2E1 mRNA. Cytochrome P-450 2E1 activity returned to normal after 3 days of daily injections. In contrast, cytochrome P-450 2B mRNA increased following a single intraperitoneal injection of 1060 mg/kg bwt and remained elevated after 3 daily injections. Cytochrome P-450 2B proteins were increased about 30-fold after a single injection and remained elevated through 3 daily injections. Cytochrome P-450 2C11 protein was rapidly suppressed (Backes *et al.*, 1990, 1993; Koop and Laetham, 1992; Sequeira *et al.*, 1992, 1994; Gut *et al.*, 1993; Yuan *et al.*, 1995 and 1997 a and b; Bergeron *et al.*, 1999). Early studies that led to these conclusions were conducted by Toftgård and Nilsen (1982), Elovaara *et al.* (1985), and Pyykko *et al.* (1987).

Exposure of Sprague-Dawley rats to 300 ppm (1302 mg/m³) ethylbenzene vapor from 1 to 3 days (6 hours/day) resulted in a 32% decrease of ethylbenzene in both blood and liver between these 2 time points (Pedersen and Schatz, 1998; 1999). Hepatic cytochrome P-450 2B1 activity (with corresponding protein levels) increased about 2-fold between 1 and 3 days; cytochrome P-450 2E1 and 1A1 activity also increased but to a lesser extent, and cytochrome P-450 2C11 protein levels decreased by Day 3. *In vitro* metabolic rates of ethylbenzene to 1-phenylethanol were increased slightly (19%) in rats exposed to ethylbenzene for 3 days compared to unexposed rats. Thus, cytochrome P-450 2B1 is not likely a major metabolizer of ethylbenzene at substrate concentration employed in this study (Pedersen and Schatz, 1998).

In a follow-up study, levels of ethylbenzene in rat lung tissue were quantified and were also found to decrease 48% from 1 to 3 days of exposure (Pedersen and Schatz, 1999). Activity of cytochrome P-450 isozymes decrease in the lung, in contrast to the liver where the cytochrome P-450 isozymes were found to increase with exposure. Cytochrome P-450 2B1 and 4B1 activity were decreased, while cytochrome P-450 2E1 was unchanged. *In vitro* metabolic rates of ethylbenzene to 1-phenylethanol were significantly decreased in lung tissue in rats exposed to ethylbenzene for 3 days compared to unexposed rats. It appears that in rats the hepatic metabolism of ethylbenzene dominates clearance of ethylbenzene following inhalation exposure, even in respiratory tissue.

Changes in liver and kidney metabolic enzymes were examined in an inhalation study conducted in male Wistar rats exposed to ethylbenzene vapor for 6 hours/day, 5 days/week at concentrations of 0, 50, 300 or 600 ppm (0, 217, 1302 or 2604 mg/m³) for 2, 5, 9, or 16 weeks. (Elovaara *et al.*, 1985). After 16 weeks exposure, NADPH-cytochrome reductase and UDPG-transferase were significantly elevated at 300 and 600 ppm. At all exposure levels aminopyrine N-demethylase and 7-ethoxycoumarin O-deethylase were elevated. Electron microscopy showed changes in hepatocyte ultrastructure as indicated by smooth endoplasmic reticulum proliferation, slight degranulation and splitting of rough endoplasmic reticulum, and enlarged mitochondria at all exposure levels beginning 2 to 9 weeks after exposure. Necrosis was not observed nor were there any increases in serum alanine aminotransferase (serum alkaline phosphatase was not measured). The proliferation of smooth endoplasmic reticulum is consistent with enzyme induction. At 16 weeks, changes in ultrastructure were mainly confined to the 600 ppm group. Hepatic glutathione content was unaffected by exposure. In the kidney, significant increases in relative kidney weight were noted following 2 and 9 but not at 16 weeks of exposure to 600 ppm. Kidney 7-ethoxycoumarin O-deethylase and UDPG-transferase activities showed statistically significant and exposure-related increases at all exposure levels.

Stott *et al.* (2003) evaluated microsomal enzyme activities in rat kidney and mouse liver and lung in concert with a number of other parameters in order to assess early target organ responses that may contribute to tumor development in these tissues. The exposures for this study consisted of 750 or 75 ppm (3255 or 325 mg/m³) ethylbenzene vapor 6 hours/day, 5 days/week administered for 1 or 4 weeks to male and female Fischer 344 rats and B6C3F1 mice. The rat kidneys and mouse liver and lungs were evaluated for changes in mixed function oxygenases and glucuronosyl transferase activities. The male rats exhibited modest induction of mixed function oxygenase and glucuronosyl transferase activities, primarily following 1-week exposure suggestive of an adaptive response to the metabolic load of ethylbenzene and its metabolites. In contrast to males, female rat kidneys exhibited minimal decreases in all mixed function oxygenase activities following 4 weeks of exposure suggesting an alteration or loss of the mixed function oxygenase competent cells in female kidney with increasing exposure period. Exposure to 75 ppm ethylbenzene for one week caused few changes to the rat kidney. A number of treatment-related changes were found in enzyme activities in the mouse tissue. In general, increases in mixed function oxygenase activities were seen in mouse liver at 1 and 4 weeks exposure; whereas the lung activities were generally decreased following one week of exposure and increased and decreased for males and females, respectively, following 4 weeks of exposure.

A study using human liver microsomes evaluated the enzyme kinetics of the initial hydroxylation reaction of ethylbenzene to 1-phenylethanol (Sams *et al.*, 2004). The production of 1-phenylethanol in hepatic microsomes exhibited biphasic kinetics with a high affinity, low Km, component and a low affinity, high Km, component. The study results indicated that cytochrome P-450 2E1 was the isoform that catalyzed the high-affinity component and cytochrome P-450 1A2 and 2B6 were likely involved in catalyzing the low-affinity component. The authors concluded that cytochrome P-450 2E1 is the major enzyme responsible for high-affinity side chain hydroxylation of ethylbenzene in human liver microsomes.

Cytochrome P-450 induction by ethylbenzene and other alkylbenzenes result in changes in the metabolism of other chemicals. Intraperitoneal injection of 1060 mg/kg bwt/day ethylbenzene for 3 days to male Holtzman rats increased the overall metabolism of toluene and shifted the oxidation from almost exclusively on the side chain to more ring hydroxylation (Sequeira *et al.*, 1992).

The pituitary glands may play a role in the regulation of cytochrome P-450 metabolism of ethylbenzene in rats. In hypophysectomized rats, the half-life for ethylbenzene in the blood was increased from 8 to 14 hours. Hypophysectomy of untreated rats caused a 50% reduction in cytochrome P-450 2C11 protein, which was restored to control levels by growth hormone supplementation. Ethylbenzene did not further suppress the level of cytochrome P-450 2C11 in hypophysectomized rats, and supplementation with growth hormone only partially restored the level. After a single exposure to ethylbenzene in intact rats, the level of cytochrome P-450 2B mRNA was significantly increased, but returned to normal after three days of exposure. In hypophysectomized rats, cytochrome P-450 2B mRNA was increased after a single exposure and remained increased after 3 exposures (Serron *et al.*, 2001).

7.3.5. Toxicokinetics

Freundt and coworkers (Freundt *et al.*, 1989, Römer *et al.*, 1986) measured EB in the blood of female Sprague-Dawley rats exposed to ethylbenzene by inhalation for two hours: Average blood concentrations of 22.5, 25.3, 68.7, 104.5, and 260 μM were determined for exposures to 120, 180, 240, 350, and 650 ppm, respectively.

A 2-week repeated-dose inhalation toxicokinetic study was conducted in which Fischer 344/N rats and B6C3F1 mice received 6-hour whole-body inhalation exposures to 75 or 750 ppm (325 or 3255 mg/m^3) ethylbenzene (Moore *et al.*, 1998; Fuciarelli, 2000). At the end of exposure (precise time not stated) on days 1, 4, and 12, whole blood, mesenteric fat, liver, and lung samples were collected to determine ethylbenzene tissue concentrations and cytochrome P-450 concentrations. Following exposure on exposure day 12, an elimination study was conducted in which whole blood, liver, lung, and mesenteric fat samples were collected, as well as urine samples collected continuously over 48 hours.

Tissue ethylbenzene concentrations were significantly higher in animals from the 750 ppm-exposed group as compared to those from the 75 ppm-exposed group for both species and sexes. Similarly, significantly higher concentrations of ethylbenzene were found in mesenteric fat compared to all other tissues. A dose-dependent, sex-related and species-specific, difference in ethylbenzene accumulation in mesenteric fat was also readily apparent. At 75 ppm, males of both species generally accumulated more ethylbenzene in mesenteric fat than females. At 750 ppm, female rats generally accumulated higher levels of ethylbenzene than male rats, and female mice accumulated lower levels of ethylbenzene than male mice.

There were no significant sex-related differences in rats in overall hepatic cytochrome P-450 concentrations as a function of either exposure concentrations or days of exposure. Overall,

hepatic cytochrome P-450 concentrations in mice generally tended to increase as a function of both exposure concentration and days of exposure.

Compared to animals receiving 750 ppm ethylbenzene, tissue ethylbenzene concentrations in animals exposed to 75 ppm were substantially lower with significantly higher individual variability. In rats, ethylbenzene exhibited bi-exponential elimination kinetics from whole blood, mesenteric fat, and lung tissue. In mice exposed to 750 ppm, bi-exponential elimination kinetics were generally observed for whole blood, mesenteric fat, and liver. However, elimination in lung tissue was mono-exponential for the 750 ppm mice.

The initial ethylbenzene tissue concentrations (C_0) and postexposure areas under the blood concentration vs. time curve (AUC_{∞}) were substantially greater in mesenteric fat samples collected from either species compared to whole blood, liver or lung tissues in the 750 ppm exposure groups. Generally in rats, the initial and terminal elimination phase half-lives for ethylbenzene were not significantly different in the tissues examined. For mice (both sexes) from the 750 ppm group, the initial elimination half-lives for liver and lung tissue, or for whole blood and fat, were not significantly different from each other; but the initial elimination half-lives for whole blood and mesenteric fat were significantly longer than those observed for liver and lung.

Significant increases in C_0 in whole blood were observed as a function of exposure concentration and females generally had significantly higher values than males in both species. There were no significant differences in the initial and terminal elimination phase half-lives between sexes for either species. However, initial and terminal elimination phase half-lives were shorter for mice as compared to rats. Saturation of ethylbenzene metabolism was suggested by non-linear (dose-dependent) toxicokinetic behavior at 750 ppm. The AUC_{∞} /exposure concentration for female rats and mice was significantly higher than for males suggesting that males were better able to eliminate (as suggested by the 75 ppm group), or alternatively accumulate less (as suggested by the 750 ppm group), ethylbenzene in circulating blood than females.

Measurable amounts of hippuric acid were detected in the urine collected from unexposed rats, with no significant difference in the 75 ppm-exposed rats, but significantly higher concentration in the rats exposed to 750 ppm ethylbenzene. Female rats (both unexposed and exposed) generally eliminated significantly higher concentrations of hippuric acid as compared to male rats. A dose-dependent increase in mandelic acid concentrations normalized to either urine volume or creatinine concentrations was readily apparent following exposures to 75 or 750 ppm ethylbenzene (no measureable mandelic acid in unexposed rats). There were no sex-related differences in the total amount of mandelic acid excreted over the 48-hr collection period.

A physiologically-based pharmacokinetic (PBPK) model for a ternary mixture of toluene, *m*-xylene, and ethylbenzene was developed by Tardif *et al.* (1997) for rats and humans using concentrations of ethylbenzene in blood collected after inhalation exposure (Tardif *et al.*, 1996) of male Sprague-Dawley rats. The approach involved the development of the mixture PBPK model in the rat and extrapolation to humans by substituting rat physiological

parameters and blood:air partition coefficients in the model with those of humans, scaling maximal velocity for metabolism on the basis of body weight^{0.75} and keeping all other model parameters species-invariant. The PBPK model adequately simulated the time course of the venous blood concentration of toluene, *m*-xylene, and ethylbenzene in rats exposed to a mixture containing 100 ppm each of these solvents. It was then scaled to predict the kinetics of toluene, *m*-xylene, and ethylbenzene in blood and alveolar air of human volunteers exposed for 7 hours to a combination of 17, 33, and 33 ppm, respectively.

As part of a two-generation inhalation reproductive toxicity study (Faber *et al.*, 2006, described below, Section 7.6) blood was collected from select dams and pups to provide information on the internal doses of ethylbenzene. On lactation day 4, individual blood samples were collected 1 hour after the third gavage dose via tail vein from four F₁ dams/group. Blood samples were collected and pooled (by litter) from culled F₂ pups from the litters of sampled dams. Blood samples also were collected from the same 4 F₁ dams 1 hour following a 6-hour inhalation exposure on *post-partum* day 22, and from 4 male and 4 female F₂ weanlings (1/sex/litter of those dams) 1 hour following completion of a single 6-hour inhalation exposure on PND 22. Blood samples were frozen and shipped to the University of Montreal for determination of whole blood ethylbenzene levels (via assay of vial head space) using a flame ionization gas chromatography method, with a detection limit of 6 µg/L.

The blood ethylbenzene analyses following oral gavage administration (26 to 342 mg/kg bwt/day) showed measurable concentrations of ethylbenzene in the dams' blood that ranged from 0.33 to 21.85 mg/L. The maternal ethylbenzene blood levels increased with increasing gavage dose in a greater than dose-proportional manner. No ethylbenzene, however, was detected in the blood samples from PND 4 pups indicating that the actual concentration was below the limit of detection (0.006 mg/L). This could be a result of either a greater elimination rate or lower milk intake rate in pups, or alternatively due to lower milk ethylbenzene concentrations. The latter explanation very likely accounts for the lower rate of transfer via milk and non-detectable concentrations in pups. The ethylbenzene concentrations in blood measured 1 hour following inhalation exposure was detected both in dams and PND 22 pups. The maternal ethylbenzene levels increased with increasing exposure in a greater than a dose-proportional manner. The concentrations of ethylbenzene in dams from the mid- and high-exposure groups were somewhat lower than those predicted by the female rat PBPK models: (measured/predicted) 11 mg/L /19 mg/L for 500 ppm, 0.56 mg/L/0.94 mg/L for 100 ppm. The magnitude and direction of the difference, however, are consistent with the expectations based on the difference between the normal adult female rats and lactating dams (i.e., difference in fat content, enzyme induction, and elimination via milk). Lower mean ethylbenzene blood levels were noted in each group after inhalation exposure than following gavage dosing. On PND 22, F₂ pup blood concentrations increased with increasing exposure levels, also in a greater than dose-proportional manner. At this developmental stage, mean pup blood levels were lower than maternal concentrations following 25 and 100 ppm exposures, but similar following exposure to 500 ppm ethylbenzene. No apparent sex differences in ethylbenzene blood levels were noted in pups at any exposure concentration.

A PBPK model for male F344 rats was developed using closed-chamber gas uptake data (Dennison *et al.*, 2003). Partition coefficients previously reported by Tardif *et al.* (1997) for Sprague-Dawley rats were used by Dennison *et al.* (2003). The Tardif *et al.* (1997) and Dennison *et al.* (2003) models have similar values for the maximal rate of metabolism, but the Michaelis constants (KMs) are very different. These models and their fit to various rat data sets are further discussed in Appendix P.

Subsequently, a PBPK model for inhaled ethylbenzene has been developed for the mouse by Nong *et al.* (2007). The initial model included published mouse physiological parameters, blood-air and tissue-air partition coefficients that were derived by the laboratory using mouse tissue and the vial equilibration technique, and the Michaelis affinity constant and maximal velocity for hepatic metabolism data of Tardif *et al.* (1997) for rats. The model was evaluated against measured blood ethylbenzene levels collected from mice exposed to 75 to 1000 ppm (325 to 4340 mg/m³) ethylbenzene for 4 hours (Charest-Tardif *et al.*, 2006). This model, however, failed to predict the blood kinetics of ethylbenzene exposure and analysis of the data indicated that hepatic metabolism (however large) and exhalation considered together were inadequate to describe the overall elimination kinetics of ethylbenzene in mice. Consequently, the mouse model was revised to include saturable metabolism in the lungs and, with this change, the model successfully predicted the inhalation pharmacokinetics of ethylbenzene in mice exposed for 6 hours to 75 or 750 ppm.

In summary, the disposition of ethylbenzene in animals and humans has been well characterized. Ethylbenzene is well absorbed from the skin, lungs, and gastrointestinal tract, rapidly distributed in the body, metabolized primarily via hydroxylation of the 2 carbons of the side-chain and then further oxidized to a range of metabolites that are excreted principally in the urine. Differences are apparent between animal species and sexes in aspects of metabolism and overall clearance of ethylbenzene. Although there are no guideline studies available for ethylbenzene metabolism and kinetics, the multitude of available experimental studies provide sufficient information to characterize ethylbenzene's metabolism and kinetics and to support PBPK models of ethylbenzene. Therefore additional metabolism and kinetic studies for ethylbenzene are not warranted.

7.4 Gene Mutation and Cytogenetics (Tiers 1 and 2)

Ethylbenzene has been extensively tested for toxicity to genetic material. Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies.

Ethylbenzene did not produce an increase in micronuclei or any signs of clastogenicity in the peripheral blood of B6C3F1 mice that inhaled up to 1000 ppm (4340 mg/m³) vapor 6 hours/day, 5 days/week for 13 weeks (NTP, 1999). The group size at study termination was 8-10 male and female in each group. At least 2000 polychromatic erythrocytes (PCEs) and 10000 normochromatic erythrocytes (NCEs) were scored. There were no effects on either micronucleated PCEs or the PCE to NCE ratio in this study.

Similarly NMRI mice that received 2 intraperitoneal injections (over 2 days) of up to 645 mg/kg bwt ethylbenzene were negative for micronuclei induction (Mohtashamipur *et al.*, 1985). The top dose was equivalent to 70% of the LD₅₀. Five animals were treated per concentration, and 1000 PCEs from the femoral bone marrow were scored from each animal. No increases in micronuclei were seen at any dose.

B6C3F1 mice that received a single 6-hour inhalation exposure of ethylbenzene vapor (500 and 1000 ppm / 2170 and 4340 mg/m³ administered to males, 375 and 750 ppm / 1627 and 3255 mg/m³ administered to females), did not exhibit in liver cells induction of DNA repair as measured by unscheduled DNA synthesis (UDS) (Clay, 2001). The author reported no significant increases, compared to the vehicle control, in mean net nuclear grain count, or in percentage of cells in repair, at either dose level in either sex. There was no evidence from this experiment for a potential to induce UDS in mouse hepatocytes. The exposure levels for each sex were based on a preliminary study that determined these exposure levels to be the maximum tolerated dose based on observed patterns of clinical signs and lethality.

In addition, ethylbenzene did not cause an increase in spontaneous recessive lethal mutations in *Drosophila melanogaster* (Donner *et al.*, 1980). This study, however, is poorly reported and hence cannot be considered a reliable assessment.

Overall, the data available on the *in vivo* genotoxicity studies indicates there is no evidence for genotoxic potential *in vivo* from well-performed tests measuring the induction of micronuclei and UDS using inhalation route of exposure. Because ethylbenzene is readily absorbed from the lungs and distributed throughout the body (IARC Monograph, 2000), ethylbenzene studies conducted by the inhalation route provide are valid for evaluation of systemic genotoxic potential of this chemical.

Ethylbenzene has consistently been found to be non-mutagenic in bacteria and yeast. Ethylbenzene has been tested in 4 *Salmonella typhimurium*/mammalian microsomal (Ames) assays up to a toxic dose of 3180 µg/plate and 3 other bacterial mutagenicity assays (2 in *Sacharomyces cerevisiae* and 1 in *E. coli*) with and without activation at dose levels up to 2000 µg/plate with negative response (Dean *et al.*, 1985; Florin *et al.*, 1980; Nestmann *et al.*, 1980; Zeiger *et al.*, 1992; Nestmann and Lee, 1983).

Ethylbenzene has produced variable mutagenic responses in the mouse lymphoma assay. In the first mouse lymphoma forward mutation assay that was conducted, ethylbenzene was mutagenic only at the highest non-lethal concentration (80 µg/mL). At this concentration, there was significant cytotoxicity with the relative total growth in 2 trials being 34 or 13% of the control level (McGregor *et al.*, 1988). In this study, ethylbenzene was not tested in the presence of S9. In the follow-up study (Wollny, 2000), 3 trials were performed with and without metabolic activation. In the first trial without activation, the results indicated a “definitive positive” at 34 and 69 µg/mL. In the same trial with activation, there was a limited positive response at 825 µg/mL. The relative growth (RTG: an indicator of cytotoxicity) with S9 mix was 18%, which is almost out of the acceptable range for this assay. In addition, positive responses were obtained in both large and small colonies, and thus both gene and chromosome mutations contributed to the response. In the 2nd and 3rd

trials, both with and without activation, the results were determined to be an inconclusive or negative, due either to insufficiently high dose levels or to an inadequate positive control response. The protocol used by Wollny (2000) for the mouse lymphoma assay is not a standard protocol. The 3-day expression period used in this study is a subject of criticism as a suboptimal protocol. The standard protocol (the International Work Group) recommended for this assay calls for 2 day expression period in contrast to the 3 day expression period used in the reported study. Overall the experiments of McGregor *et al.* (1988) and Wollny (2000) indicate a positive and an ambiguous mutagenic effect of ethylbenzene in L5178Y tk+/- mouse lymphoma cells. It should be noted that ethylbenzene induces mutations in this assay over a very narrow window, generally associated with doses near the cytotoxicity limit. The relevance of such a response is difficult to understand in view of other negative genetic toxicology results.

A mouse lymphoma forward mutation assay was recently performed according to OECD Guideline 476, USEPA OPPTS 870.5300 and EC, B.17 and this study found ethylbenzene to be non-mutagenic in the absence and presence of metabolic activation based on results of the initial and confirmatory mutagenicity assays. (Seidel *et al.* 2006). This study included the results of three assays: preliminary toxicity assay; initial mutagenicity assay and confirmatory assay. Based upon the results of the toxicity assay, concentrations in the range of 10 to 120 µg/mL were selected for the initial mutagenicity assay both in the absence and presence of S9. There was no increase in mutant frequency above 95×10^{-6} , the average of the concurrent solvent control, and there was no positive dose related linear trends at any concentrations of ethylbenzene evaluated in this assay with or without metabolic activation. In the initial mutagenicity assay in the absence of S9, cultures treated with ethylbenzene showed excessive toxicity at concentration levels from 60 to 120 µg/mL. In the presence of S9, cultures treated with ethylbenzene displayed excessive toxicity at concentrations of 80, 100 and 120 µg/mL. In the confirmatory mutagenicity assay, 54, 60 and 70 µg/mL indicated excessive toxicity in the absence of S9. In the presence of S9, cultures treated with 90 µg/mL of ethylbenzene displayed excessive toxicity. All other mutant plates from the remaining treatments were evaluated. Based upon results of the initial and confirmatory mutagenicity assays, ethylbenzene was considered to be non-mutagenic in the absence and presence of metabolic activation in this *in vitro* mouse lymphoma (L5178Y tk+/-) forward mutation assay.

Negative results were reported in an *in vitro* study of chromosome aberrations in rat liver cells (Dean *et al.*, 1985) and in Chinese hamster ovary (CHO) cells in the absence or presence of metabolic activation (NTP, 1999). Cultures were treated with concentrations up to 125 µg/mL in both metabolic conditions. Although cytotoxicity values corresponding to each dose were not given, the higher dose level of 150 µg/mL was toxic as indicated by finding no metaphases to score.

An *in vitro* sister chromatid exchange assay conducted for ethylbenzene using CHO cells was negative in the presence or absence of metabolic activation (NTP, 1999). Norppa and Vainio (1983) reported a marginally positive sister chromatid exchange response in human whole blood lymphocytes at the highest toxic dose (10 mM) after incubation with ethylbenzene for

48 hours (concentrations ranged from 0.1 to 10 mM). However, this study cannot be considered reliable since the study protocol has not been validated.

An *in vitro* Syrian hamster embryo micronucleus assay was positive (Gibson *et al.*, 1997). The micronucleus test involved the exposure of the cells in the presence of cytochalasin B for 24 hours before harvesting and processing for micronuclei evaluation. 500 Cells were counted to determine the number of binucleated cells and 1000 cells per concentration analyzed for micronuclei. Doses up to 200 µg/mL were used, with the top dose causing a greater than 50% reduction in cell number and reduction of binucleate cells from 48% to 16%. Statistically significant increases, corresponding to a greater than 2-fold increase in micronucleated cells, were seen at all doses, including those not associated with high toxicity, indicating a positive response for this assay.

In Syrian hamster ovary cells, ethylbenzene (up to 500 µg/mL) did not induce cell transformations in a 24 hour period. Cell transformations, however, did occur after a 7-day incubation period at ethylbenzene concentrations of 150 to 200 µg/mL. It was suggested by the authors that chemicals that are positive at 7 days, but negative after 24 hours act by (or through) a promotion-like mechanism (Kerckaert *et al.*, 1996; Hazleton, 1995a,b). This conclusion requires reevaluation in light of a positive response in the micronucleus assay in these cells.

The overall conclusion on mammalian cell assays is that ethylbenzene does not induce sister chromatid exchange or clastogenicity in these assays. There is some evidence that ethylbenzene induces gene mutations in mammalian cells but the effect is difficult to reproduce as it occurs only over a very small dose range and has been shown in two independent studies not to be reproducible at similar concentrations. It is difficult to evaluate the significance of such a response and it may therefore be concluded that the potential for ethylbenzene to be mutagenic in the mouse lymphoma assay is equivocal. A recent mouse lymphoma assay performed according to current standards, however, found a negative mutation result for ethylbenzene. Ethylbenzene was positive *in vitro* in the SHE cell transformations and micronucleus assays.

Overall, the data available on the *in vivo* genotoxicity studies indicates there is no evidence for genotoxic potential *in vivo* from well-performed tests measuring the induction of micronuclei and UDS using inhalation route of exposure.

In summary, ethylbenzene has been tested in a wide battery of mutagenicity and chromosome assays and the weight-of-the-evidence is that ethylbenzene is not genotoxic. No further evaluation of ethylbenzene's genotoxic potential is warranted.

7.5 Subchronic Studies (Tier 2)

Ethylbenzene has been well characterized for subchronic toxic effects with studies available in several animal species for inhalation and oral exposure. Overall, relatively high exposures were required to produce toxic effects with target findings consistently noted in the liver and kidney.

In a 4-week study, Fischer 344 rats and B6C3F1 mice (5/sex/dose) were exposed by inhalation to 0, 100, 400, or 800 ppm (0, 434, 1736, or 3472 mg/m³) ethylbenzene, six hours/day, five days/week (Cragg *et al.*, 1989). There were no effects on survival, body weight gain, clinical chemistry, and gross or microscopic pathology. For the rats, exposure to 800 ppm resulted in an approximate 20% and 13% increase in relative (to body weight) liver weights in females and males, respectively. Female rats that received 400 ppm ethylbenzene exhibited about a 7% increase in relative liver weight; whereas, the male relative liver weights at this exposure concentration were not significantly different from controls. In the mice that received 800 ppm ethylbenzene, liver weights relative to body weight were not statistically significantly different in males or females; but absolute liver weight was increased in females (about 15%), and liver weights relative to brain weights were increased in males (about 17%) and females (about 15%). The authors interpreted the liver changes as probably metabolic adaptation, due to the absence of liver histopathology or abnormal clinical chemistry. The results of this study support a subchronic NOAEL of 800 ppm for rats and mice. In this same study, New Zealand White rabbits (5/sex/group) received 4-week exposures to 0, 400, 800, or 1600 ppm (0, 1736, 3472, or 6944 mg/m³) ethylbenzene. The effects in rabbits were limited to a transient initial decrease in body weights following exposure to 1600 ppm; thus the study NOAEL for rabbits was 1600 ppm ethylbenzene.

The U.S. National Toxicology Program (NTP, 1992a) conducted 13-week inhalation studies in rats and mice. Male and female Fischer 344 rats (10/sex/dose) were exposed up to 1000 ppm (4340 mg/m³) ethylbenzene 6 hours/day, 5 days/week for 13 weeks (NTP, 1992a). There was a slight decrease (5 to 7%) in body weight in both sexes, which was not statistically significant. Absolute and/or relative liver, kidney, and lung weights occurred at 250 ppm (1086 mg/m³) and higher. Chemically-related histopathological changes were not observed in any tissues. Inflammatory changes in the lung corresponded with the increases in lung weights but the occurrence and severity of these changes suggested they were probably unrelated to ethylbenzene. Since the liver and kidney weight changes occurred in the absence of histopathological changes, these findings were not considered adverse and the NOAEL was considered to be 1000 ppm.

Male and female B6C3F1 mice (10/sex/dose) were exposed up to 1000 ppm (4340 mg/m³) ethylbenzene six hours/day, five days/week for 13 weeks (NTP, 1992a). No adverse effects were reported for survival, body weights, or treatment-related pathological findings. Increased liver weights occurred in both sexes in exposed groups at 750 (3225 mg/m³) and 1000 ppm, and increased kidney weights in females at 1000 ppm. Since the organ weight changes occurred in the absence of histopathological changes, these findings are not considered adverse supporting a study NOAEL of 1000 ppm.

Groups of Wistar rats (18/sex/dose) were exposed to 0 or 100 ppm (434 mg/m³) ethylbenzene 6 hours/day, 5 days/week, for 12 weeks (Clark, 1983). Assessments were made of survival, clinical observations, body weight, feed intake, hematology, clinical chemistry, urinalysis, organ weights, and histopathology of all major organs. There were no statistically significant effects observed at 100 ppm. No differences from controls were noted in liver enzymes including serum alkaline phosphatase. Slight bile duct hyperplasia was seen in 15/18

exposed males and 14/18 exposed females, however these observations were not statistically significant and hyperplasia was also common in control animals (10/18 females and 8/18 males). The results of this study support a NOAEL of 100 ppm.

Liver and kidney effects were confirmed and further defined in an inhalation study conducted in male Wistar rats (Elovaara *et al.*, 1985). In this study, groups of male rats (5/group) were exposed for 6 hours/day, 5 days/week to ethylbenzene concentrations of 0, 50, 300, or 600 ppm (0, 217, 1302 or 2604 mg/m³) for 2, 5, 9, or 16 weeks. The liver was the only organ examined histologically (light and electron microscopy). There were no changes in liver weight at any concentration. After 16 weeks exposure, NADPH-cytochrome reductase and UDPG-transferase were significantly elevated at 300 and 600 ppm. At all exposure levels aminopyrine N-demethylase and 7-ethoxycoumarin O-deethylase were elevated. Electron microscopy showed changes in hepatocyte ultrastructure as indicated by smooth endoplasmic reticulum proliferation, slight degranulation and splitting of rough endoplasmic reticulum, and enlarged mitochondria at all exposure levels beginning 2 to 9 weeks after exposure. Necrosis was not observed nor were there any increases in serum alanine aminotransferase (serum alkaline phosphatase was not measured). The proliferation of smooth endoplasmic reticulum is consistent with enzyme induction. At 16 weeks, changes in ultrastructure were mainly confined to the 600 ppm group. Hepatic glutathione content was unaffected by exposure. In the kidney, significant increases in relative kidney weight were noted following 2 and 9 but not at 16 weeks of exposure to 600 ppm. Kidney 7-ethoxycoumarin O-deethylase and UDPG-transferase activities showed statistically significant and exposure-related increases at all exposure levels. In the absence of histologic evidence of damage, the changes in absolute or relative liver weight and no effect on serum alanine aminotransferase, the microsomal enzyme induction and ultrastructural changes are considered adaptive responses to ethylbenzene. The results of this study support a NOAEL of 600 ppm.

An inhalation study with exposures up to 6 months was conducted in small groups of Wistar rats, guinea pigs and rabbits, and one rhesus monkey (Wolf *et al.*, 1956). The exposure levels tested ranged from 400 ppm (1736 mg/m³) to 2200 ppm (9548 mg/m³). Slight liver effects were seen in rats, guinea pigs, and monkeys and slight kidney effects were noted in rats. Testes effects, described as degeneration of the germinal epithelium, were noted in rabbits and the monkey. The testes effects reported in this study have not been confirmed in any of the other repeated exposure studies that have been conducted in rats, mice, guinea pigs, and rabbits. The Wolf *et al.* (1956) study used very few experimental animals, variable exposure periods, and limited assessments and hence is not considered a reliable evaluation of ethylbenzene subchronic toxicity.

The subchronic oral toxicity of ethylbenzene has been assessed in a study conducted in Wistar rats (Mellert *et al.*, 2004, 2007). Rats (10/sex/dose) received gavage doses of 0, 75, 250, or 750 mg/kg bwt/day ethylbenzene administered each day as 2 part doses with an interval of about 8 hours. There were no deaths attributed to ethylbenzene treatment. Clinically, post-dose salivation was observed in ≥ 250 mg/kg bwt/day animals and discoloration of urine was noted in ≥ 750 mg/kg bwt/day animals. The salivation was likely due to local irritation of the test material to the upper digestive tract; whereas the urine finding was unexplained as no urine discoloration was seen in the urinalysis performed

towards the end of the study. Body weight decreases (about 14% below controls) and decreases in feed efficiency occurred in 750 mg/kg bwt/day males although feed consumption increased in these animals beginning on day 70. Both 750 mg/kg bwt/day males and females consumed about 45% higher amounts of water than controls. The 250 mg/kg bwt/day males also showed on some days increases in water consumption. Local irritation of the upper digestive tract probably resulted in the increased water intake. There were a number of changes in blood, clinical chemistry, and urinalysis parameters at doses \geq 250 mg/kg bwt/day. The mean corpuscular volume was enlarged in 750 mg/kg bwt/day animals and in 250 mg/kg bwt/day females. The high dose females also exhibited a decrease in platelets. Both the hematology changes were considered treatment-related and possibly due to a minimal transitional regenerative anemia. Prothrombin times were also reduced in \geq 250 mg/kg bwt/day animals of both sexes, however, the reduction in the 750 mg/kg bwt/day males occurred only as a tendency toward shorter clotting times. Changes in clinical chemistry present in 250 mg/kg bwt/day males included slight increases in liver enzymes and increases in potassium, calcium, total bilirubin, and cholesterol. The 250 mg/kg bwt/day females exhibited only higher cholesterol levels. At 750 mg/kg bwt/day, both sexes exhibited increased liver enzymes, potassium, total bilirubin, albumin, cholesterol and magnesium concentrations. The 750 mg/kg bwt/day males also had higher calcium and urea and decreased creatinine concentrations. In 750 mg/kg bwt/day females, total protein and globulin concentrations were increased and sodium levels decreased. The clinical chemistry changes were speculated to be due to induction of the hepatic microsomal enzyme system and/or secondary to effects on feed and water consumption. The urinalysis treatment findings were limited to an increase in numbers of degenerated transitional epithelial cells and granular epithelia cell casts in \geq 250 mg/kg bwt/day males. These findings indicated mild damage or functional impairment to the kidneys. The neurologic assessment was largely unaffected by ethylbenzene exposure with two exceptions. A decrease in the value of the landing foot-splay test occurred in 750 mg/kg bwt/day males that may have been related to the decrease in body weight in this group. The females in the 750 mg/kg bwt/day group exhibited increased motor activity but the finding was atypical occurring at intervals 3, 6, and 10 (of 12 intervals) suggesting an incidental and not treatment-related finding. Treatment-related pathology changes were present in the liver of both sexes and in the male kidney. In the liver, an increase in absolute and relative weights was recorded in both sexes at \geq 250 mg/kg bwt/day, which was correlated with an accompanying centrilobular hypertrophy in the majority of animals, indicating an adaptive reactive response of the liver. No compound related toxic changes were detected in the liver. In the kidneys of \geq 250 mg/kg bwt/day males, an increase in absolute and relative weights and in hyaline droplets in the tubular epithelium was noted. The increase in hyaline droplets was considered as an increase of the male specific protein α -2u-globulin. The \geq 250 mg/kg bwt/day females also exhibited slight increases in kidney weights that was not correlated with microscopic changes and hence was considered of no biological relevance. The \geq 250 mg/kg bwt/day females also showed a decrease in thymus weights but this change was not correlated with histopathological changes and hence was considered of no biological relevance.

Subchronic toxicity testing in rodents consistently found the liver and kidney as targets for ethylbenzene effects. The primary findings were increases in organ weights that generally occurred without evidence of structural changes (on gross and routine microscopic

examination). One study that evaluated ultrastructure (by electron microscopy) of the liver cells of male rats that received inhalation exposure to ethylbenzene vapor from 2 to 16 weeks found proliferative changes in smooth endoplasmic reticulum (Elovaara *et al.*, 1985), which the authors' concluded was consistent with enzyme induction. Additionally, a number of studies (*e.g.* Backes *et al.*, 1990, 1993; Koop and Laetham, 1992; Sequeira *et al.*, 1992, 1994; Gut *et al.*, 1993; Yuan *et al.*, 1995; Yuan *et al.*, 1997a,b; Bergeron *et al.*, 1999; Stott *et al.*, 2003) have demonstrated metabolic enzyme or enzyme activity changes associated with ethylbenzene exposure. Thus, as the liver and kidney have significant metabolic activity and ethylbenzene is a substrate for enzymatic metabolism, it is not unexpected that ethylbenzene would in some instances cause an induction of the metabolic enzymes and increase the substance of these tissues. Not known is what, if any, toxicological significance may arise from these changes. As metabolic enzymes also metabolize endogenous substances, changes may indeed be toxicologically important, but within the assessments of a standard subchronic toxicity study, these type effects occurring with just slight to moderate increases in organ weight would not likely be discernable. For ethylbenzene, however, there are chronic toxicity studies available (described in the Chronic Toxicity/Carcinogenicity Section) that provide a further indication on target organ effects with continued exposure that are helpful in retrospective consideration of the subchronic ethylbenzene study findings. These studies do not support the subchronic rat liver or mouse kidney effects as toxicologically important as these organs did not demonstrate pathology following chronic exposure. Alternatively, as chronically, ethylbenzene produced pathological lesions in the rat kidney and mouse liver, then the subchronic effects seen in these organs may have some toxicological role in the eventual development of chronic rat kidney and mouse liver effects. The relationship of the subchronic liver and kidney weight changes to chronic toxicity findings in these organs, however, can not be known with certainty, hence the conclusion of this assessment is that these organ weight changes are of questionable toxicological significance. For the purposes of identifying study effect levels for ethylbenzene in the subchronic studies, the organ weight changes absent toxic pathology were considered as "effect" levels but not as "adverse effect" levels as these changes did not appear to adversely impact the health and well-being of the animals on study. The longer-term studies therefore may be more informative on repeated exposure target organ effect in rodents than can be discerned from the available subchronic toxicity studies.

There are additional target effects of note from subchronic exposures of rodents to ethylbenzene. The subchronic oral toxicity study of ethylbenzene found more pronounced body weight and organ weight changes than did the subchronic inhalation studies and, in addition, found an array of changes in clinical chemistry enzymes, minerals, and electrolytes. These changes were thought to have arisen secondary to the effects on the liver and kidney. In addition, hematology changes were found in the subchronic oral study suggestive of a regenerative anemia. These changes are not explained by the kidney pathology as non-regenerative anemia is the form of anemia observed with severe renal disease. Since no changes were observed in the other blood cell parameters at the end of the study, the authors concluded that the anemic process may have occurred at the beginning of the study. Prothrombin times were also reduced in the ethylbenzene treated animals. The biological significance of this change is unknown as generally prolonged times (and not shorter times) are observed with diseases that affect this blood parameter. Although, there was some

evidence of a reduction in platelet counts, this occurred only significantly in one sex and without a clear dose response relationship. In addition, in the many repeated-exposure studies that have been conducted for ethylbenzene, including chronic studies and studies with high exposure concentrations, there have been no reports of hypercoagulation disorders associated with ethylbenzene exposure.

As ethylbenzene has available subchronic inhalation and oral studies in multiple animal species in addition to chronic inhalation studies in rats and mice (discussed below), no further testing of ethylbenzene subchronic toxicity is needed.

7.6 Reproduction and Fertility Effects (Tiers 1 and 2)

For the VCCEP program, a rat 2-generation inhalation reproductive toxicity study was sponsored by the American Chemistry Council Ethylbenzene Panel and conducted at WIL Research Laboratories, Inc (Stump, 2004a, Faber *et al.*, 2006). Four groups of male and female Crl:CD[®](SD) IGS BR rats (F₀ generation: 30/sex/group; F₁ generation: 25/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, at 0, 25, 100, and 500 ppm (0, 108, 434, and 2170 mg/m³) for 6 hours daily, 7 days per week. Inhalation exposure of the F₀ and F₁ females was suspended from gestation day 21 through lactation day 4 and, on lactation days 1 through 4, these females received the vehicle, corn oil, or test article in the vehicle via oral gavage at dose levels of 0, 26, 90 and 342 mg/kg bwt/day (divided into three equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg bwt/dose. The exposure concentrations in this study were selected based on the results of a pilot reproductive toxicity study (Stump, 2003) that found significant pup and weanling effects (decreased pup body weight gain, mortality and adverse clinical signs in newly weaned animals receiving inhalation exposure) at 500 and 1000 ppm ethylbenzene. The oral dosages utilized in this study were calculated to produce equivalent doses to the inhalation exposures using the ethylbenzene PBPK model (Tardif *et al.*, 1997). One litter was produced in each generation. Assessments were made of the F₀ and F₁ animals for gonadal function, estrous cyclicity, spermatogenic endpoints, mating behavior, conception rate, gestation, parturition, lactation and weaning, F₁ and F₂ generation offspring growth and development, and gross and microscopic pathology of select tissues including reproductive organs. In addition, a developmental neurotoxicity assessment was included in this study (described separately below) to assess potential adverse functional and/or morphological effects in the F₂ offspring following F₁ generation exposure and blood was collected from select dams and pups to provide information on internal doses of ethylbenzene (described separately above).

Ethylbenzene did not deleteriously affect reproduction or offspring development in rats exposed over 2 generations. There were no test article-related deaths or clinical observations in any test article exposure group in either generation of animals. Mild effects on body weight gain were observed in male parental animals at 500 ppm during the first several weeks of the study. The body weight reductions did not persist in either generation. At necropsy, no macroscopic or microscopic findings related to test article exposure were observed at any exposure concentration. F₀ and F₁ males that inhaled 500 ppm ethylbenzene exhibited increases in absolute and relative (to final body weight) liver and kidney weights.

Relative liver weights were increased significantly in the females from both generations in the 500 ppm/342 mg/kg bwt/day group. The increase in absolute and relative liver weight has been noted in other repeated-dose toxicity studies with ethylbenzene (Cragg, *et al.*, 1989; NTP, 1992a; Mellert *et al.*, 2004, 2007) and in certain instances, has been associated with increased levels of metabolic enzymes in the liver (Elovaara, *et al.*, 1985). However, the relationship between increased liver weights and hepatic enzyme induction is not straightforward and the toxicological significance of the increased liver weights in the reproducing and developing animal is unknown.

Ethylbenzene did not adversely affect reproductive performance in either sex from F₀ or F₁ generations. Estrous cycle length, pre-coital intervals, male and female mating and fertility indices, gestation length, spermatogenic endpoints and reproductive organ weights were similar in all exposure groups. The ovarian follicle counts for the F₁ females in the 500 ppm/342 mg/kg bwt/day group were similar to the control values. There were no test article-related changes to F₁ and F₂ litter parameters including pup sex ratios, live litter sizes, number of dead pups, viability indices, pup body weights and the general physical condition of the pups. In the pilot reproductive study (Stump, 2003), dose-related decreases in offspring preweaning and postweaning body weights, as well as offspring survival immediately following weaning at postnatal day 22 occurred at exposure levels of 500 and 1000 ppm. In the current study, however, there was no exposure-related offspring mortality or preweaning body weight effects in either the F₀ or F₁ generation litters. The pre-weaning developmental landmarks pinnal detachment, hair growth, incisor eruption, and eye opening and the post-weaning developmental landmarks balanopreputial separation and vaginal patency were unaffected by ethylbenzene exposure in either the F₁ or F₂ generations. As also occurred in some of the other physical landmarks of development assessed in the F₁ and F₂ generation offspring (e.g., eye opening and hair growth), the timing of appearance of these landmarks was not always consistent across generations in the control groups of animals and/or individual control mean values were notably different from the historical control mean for that endpoint. Therefore, the slight differences among groups, a few of which were statistically significant, were not considered to be related to parental ethylbenzene exposure.

In summary, slight body weight decreases and increased liver and kidney weights of similar magnitude were observed in the males of both generations exposed to 500 ppm of ethylbenzene. Significant increases in relative liver weights also occurred in females of both generations. The transient nature of the body weight changes and the lack of histopathological change associated with the increased organs weights suggest that these changes were not adverse. Research conducted prior to this study has demonstrated that ethylbenzene can induce metabolic enzymes and that these changes are correlated with these increased organ weights. Reproductive performance was not affected in either generation and no pattern of developmental toxicity was observed in the F₁ and F₂ offspring. Based on these results, an exposure level of 100 ppm or 100 ppm/90 mg/kg bwt/day (gavage from gestation day 21 to lactation day 4) was considered to be the NOEL (no-observed-effect level) for general parental toxicity in this study, with 500 ppm or 500 ppm/342 mg/kg bwt/day (gavage from gestation day 21 to lactation day 4) considered a NOAEL (no-observed-adverse-effect level). The NOAEL for parental reproductive toxicity and for

general developmental toxicity was considered to be 500 ppm or 500 ppm/342 mg/kg bwt/day of ethylbenzene, the highest exposure level tested in this study.

A fertility assessment for ethylbenzene was also included in a developmental toxicity conducted in rats by NIOSH (U.S. National Institute for Occupational Safety and Health)(Andrew *et al.*, 1981; Hardin *et al.*, 1981). Female Wistar rats were exposed to 0, 100, or 1000 ppm (0, 434, or 4340 mg/m³) ethylbenzene 7 hours/day, 5 days/week for 3 weeks; mated with unexposed males; and pregnant females were further exposed to 0, 100, or 1000 ppm (0, 434, or 4335 mg/m³) 7 hours/day through Gestational Day 19. Maternal effects (increased organ weights) occurred in the 1000 ppm-exposed group. A higher percentage of ethylbenzene exposed females mated (were sperm positive) than the controls (67, 78 and 74% for 0, 100 and 1000 ppm, respectively) and a slightly smaller percentage of ethylbenzene-exposed females that mated were pregnant at gestation day 21 (89, 77 and 77%, respectively). When expressed on the basis of total females per group, 56, 60, and 57% of the females exposed to 0, 100, or 1000 ppm were pregnant at gestation day 21. Thus exposure of female rats to ethylbenzene at 100 or 1000 ppm for three weeks did not decrease fertility.

No effects on reproductive organs were also reported in rats, mice, and rabbits exposed to ethylbenzene for up to 13 weeks in repeated dose studies (Cragg *et al.*, 1989; NTP, 1992a). In the 13-week NTP study (1992a), there were no treatment-related effects on sperm counts and motility, testicular morphology, length of estrous cycle, caudal or epididymal weights in rats or mice exposed to 100, 500, or 1000 ppm (0, 434, 2170, or 4340 mg/m³) ethylbenzene. An earlier study (Wolf *et al.*, 1956) reported testicular lesions in rabbits and a monkey that inhaled ethylbenzene vapors for up to 6 months; however, this study is not considered reliable as it used very few experimental animals and limited assessments.

The available information that has been collected for ethylbenzene on reproduction and fertility effects provides a thorough characterization of this health effects endpoint and overall this information demonstrates that ethylbenzene is not a reproductive hazard. No further reproductive toxicity testing of ethylbenzene is warranted.

7.7 Prenatal Developmental Toxicity (Tier 2)

Four studies have investigated the developmental toxic effects of ethylbenzene in laboratory animals.

In the earliest study conducted by NIOSH, groups of female Wistar rats were exposed by inhalation to 0, 100, or 1000 ppm (0, 434, or 4340 mg/m³) ethylbenzene, 7 hours/day for three weeks prior to mating, then 7 hours/day during 1-19 days of gestation (Andrew *et al.*, 1981; Hardin *et al.*, 1981). Some of the rats received just pregestational exposures, others received only gestational exposures, and another set received exposures during both periods. Maternal effects were observed only at 1000 ppm, and included increased liver, kidney, and spleen weight changes (approximate change of 22%, 10%, and 10%, respectively), with no accompanying histopathological effects. In the gestation only exposed rats, there was an increase incidence of supernumerary and rudimentary ribs in the 1000 ppm group and an elevated incidence of extra ribs in the 100 and 1000 ppm groups. In the rats that were also

exposed pregestationally, there was also an increased incidence of extra ribs 1000 group only. There were no increases in rudimentary ribs in these rat groups. The skeletal variants in this study are considered marginally adverse. The results of the study indicate a NOAEL/LOEL for maternal toxicity of 1000 ppm and a NOAEL for developmental toxicity of 100 ppm.

In the same study, New Zealand White rabbits exposed by inhalation to 0, 100, or 1000 ppm (0, 434, or 4340 mg/m³) ethylbenzene during days 1 to 24 of gestation had no developmental effects. Maternal effects (increased liver weights) were observed in the 1000 ppm does only but there was no accompanying evidence of histopathological changes. The NOAEL in this study for both maternal and developmental toxicity in the rabbits is 1000 ppm. (Andrew *et al.*, 1981; Hardin *et al.*, 1981).

The NIOSH study, although not conducted using the current EPA or OECD testing guidelines, had a study design that is substantially similar to current guidelines and for some parameters exceeds the current guidelines (e.g. exposure initiated on the day after impregnation, histopathology was conducted on certain maternal organs). Also, although only 2 exposure concentrations were assessed in this study, the high concentration 1000 ppm (or 4.34 mg/L) exceeded the EPA's developmental toxicity guideline limit dose of 2 mg/L.

A poorly reported developmental toxicity study was conducted using rats, rabbits, and mice by Ungvary and Tatrai (1985) and is summarized in IRIS (1991). CFY rats were exposed to 600, 1200, 2400 mg/m³ (138, 277, 553 ppm) ethylbenzene 24 hours/day from gestational days 6 to 15 or for 3 days intermittently for 4 hours/day for gestational days 6 to 16. The results from this study are unclear if they pertain to the continuous or the intermittent exposure. New Zealand rabbits were exposed for 24 hours/day to concentrations of 500 or 1000 mg/m³ (115 or 230 ppm) from gestational days 7 to 20. Controls consisted of untreated animals and those exposed only to air. Maternal toxicity (unspecified species) was reported as moderate and concentration dependent; however no confirmatory data was presented. The rabbits exposed to 1000 mg/m³ exhibited mild maternal toxicity as indicated by reduced weight gain; however the percent weight gain was not presented. There were no data for developmental endpoints in this group because there were no live fetuses. One doe died, 3 others aborted, and 4 does had total resorptions. Other test substances in this study at this same concentration all caused spontaneous abortions causing doubt on the significance of the ethylbenzene findings. The NIOSH study in rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981) also did not find any indications of abortions suggesting that the effects seen by Ungvary and Tatrai (1985) in rabbits were not treatment-related. Ungvary and Tatrai (1985) did observe a significant reduction in the mean female fetal weight in rabbit does exposed 24 hours/day to 500 mg/m³; whereas the NIOSH study (Andrew *et al.*, 1981; Hardin *et al.*, 1981) did not observe such an effect in rabbits exposed up to 4348 mg/m³. These conflicting results in rabbits might be due to differences in study design. Postimplantation loss and exposure-related skeletal retardation were significantly elevated in rats at all exposure levels with one exception. Exposure to 600 mg/m³ for 6 hours/day (not stated if this was a single exposure or the exposure duration on each day of gestation) did not result in any statistically significant fetal effects although there was an increased incidence of dead/resorbed fetuses, lower weight of fetuses, and skeletal retarded fetuses. In the 24 hour/day exposure groups, malformations described as "anomalies of the urogenital apparatus" and an increased

incidence of extra ribs were significantly increased only at the highest exposure concentration. There were no data presented on the anomalies of the uropoietic apparatus. There was a significant increase in skeletal retardation and fetal resorption in all continuous exposure groups although the concentration-response was shallow. In mice, an increased incidence of “anomalies of the uropoietic apparatus” was the only observation, but no data was reported. There was no discussion of concerning maternal toxicity in the mice. Overall there is insufficient information in the Ungvary and Tatrai study report (1985) to consider the significance of their developmental toxicity findings for ethylbenzene.

The most recently reported developmental toxicity study for ethylbenzene was conducted by Saillenfait *et al.* (2003) in rats using a study design comparable with current U.S. EPA and OECD testing guidelines. In this study, pregnant Sprague-Dawley rats were exposed by inhalation to 0, 100, 500, 1000, or 2000 ppm (0, 434, 2170, 4340, 8680 mg/m³) ethylbenzene for 6 hours/day, during days 6 through 20 of gestation. Maternal toxicity was evident as clinical signs of toxicity (ataxia, decreased motor activity) at 2000 ppm and reduced maternal body weight, body weight gain and feed consumption at 1000 and 2000 ppm. No evidence of teratogenic effects was found at any exposure level. Fetal toxicity evidenced by significant decrease in fetal body weights occurred at 1000 and 2000 ppm. These decreases amounted to 7 and 18% of the control values at 1000 and 2000 ppm, respectively. No significant differences were observed between the control and treated groups in the incidences of either individual or total external or visceral variations, or individual skeletal variations. There was an increased number of fetuses with skeletal or any variations at 1000 and 2000 ppm. The mean percentage of fetuses per litter with skeletal or any variations was also significantly increased at 2000 ppm. Summarizing these data, ethylbenzene produced developmental toxicity at 1000 and 2000 ppm, concentrations that also produced significant maternal toxicity. The results of this study indicate a NOAEL for maternal and developmental toxicity of 500 ppm.

Saillenfait *et al.* (2006) also reported on a recent developmental toxicity study conducted in rats with ethylbenzene and combined exposure to ethylbenzene and methyl ethyl ketone. In this study, pregnant Sprague-Dawley rats were exposed to ethylbenzene (0, 250, or 1000 ppm; 0, 1085, or 2170 mg/m³) and methyl ethyl ketone (0, 1000, or 3000 ppm), alone and in combination, by inhalation, for 6 hours/day, during days 6-20 of gestation. Maternal toxicity, evidenced by decreased in body weight gain and feed consumption, tended to be greater after simultaneous exposures to the high concentrations of 1000 ppm ethylbenzene and 3000 ppm methyl ethyl ketone, when compared to the treatments with individual compounds. No significant increase in embryo/fetal lethality or incidence of malformations and variations was observed in any of the treatment groups. Fetal body weight was significantly reduced after individual treatment with 1000 ppm ethylbenzene or 3000 ppm methyl ethyl ketone, and in the combined groups. There was no evidence of interaction between ethylbenzene and methyl ethyl ketone in causing developmental toxicity.

In summary, the developmental toxicity potential of ethylbenzene vapor has been thoroughly studied in two laboratory animal species. In these studies, ethylbenzene produced some evidence of developmental toxicity, but not teratogenicity, at concentrations that were

associated with maternal effects. Further developmental toxicity testing for ethylbenzene is unnecessary.

7.8 Immunotoxicity (Tier 2)

For the VCCEP program, a rat immunotoxicity study was sponsored by the American Chemistry Council Ethylbenzene Panel and conducted at WIL Research Laboratories, Inc. and ImmunoTox, Inc. (Stump, 2004b). Four groups of female Crl:CD[®](SD) IGS BR rats (10/group) were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, at 0, 25, 100, or 500 ppm (0, 108, 434, 2170 mg/m³) for 6 hours daily, 7 days per week for 28 consecutive days. A group of rats received the positive control agent, cyclophosphamide (50 mg/kg bwt/day; intraperitoneal injection), for 4 consecutive days through the day prior to the scheduled necropsy. All animals received a single intravenous immunization injection via a lateral tail vein of sheep red blood cells approximately 4 days prior to the scheduled necropsy. Female rats only were used for the study based on essentially similar toxicity profiles in males and females in subchronic toxicity studies and preference for the more docile female for the intravenous tail injection procedure. All animals were observed twice daily for clinical signs and mortality (prior to exposure and within 1 hour after completion of each exposure period) and weekly detailed physical examinations were conducted. Body weights and feed consumption were recorded twice weekly until study termination. Blood was collected for hematology evaluations from all animals at the time of the scheduled necropsy (study week 4). All animals were subjected to complete necropsies and selected organs were collected and weighed. Splenic tissues were collected from all animals at the scheduled necropsy. The splenic samples were randomized for antibody-forming cell (AFC) analysis so that the analyst was unaware of the treatment group of each sample examined. The AFC response was evaluated as either specific activity (AFC/10⁶ spleen cells) or as total spleen activity (AFC/spleen).

Ethylbenzene exposure did not adversely affect the rats' survival, clinical signs, body weight, or feed consumption. No treatment-related effects on hematology parameters were observed. As has been reported in previous studies, ethylbenzene produced increases in liver and kidney weights relative to final body weights (13% for both) in the 500 ppm group. There were no treatment-related effects of ethylbenzene on IgM antibody forming cell response. Cyclophosphamide performed as expected, exhibiting a decrease in spleen and thymus weights and a decrease in spleen cell numbers and in IgM antibody forming cell response. The results of this study support a study NOAEL of 500 ppm ethylbenzene.

There are no other specific immunotoxicity studies reported for ethylbenzene. Immune system tissues/organs, however, were included among the tissue/organs evaluated in a number of repeated exposure studies and no weight changes or microscopic lesions were detected with ethylbenzene exposure (NTP, 1992a, 1999; Mellert *et al.*, 2004; Stump, 2004a).

The findings of the 28-day rat inhalation study demonstrates that ethylbenzene at up to 500 ppm vapor concentration does not adversely affect the functional ability of the humoral component of the immune system of rats as measured by splenic IgM antibody forming cell response to the T-dependent antigen, sheep erythrocytes. Absent any evidence of adverse immunologic findings in this screening study, no further immunotoxicity testing is recommended for ethylbenzene.

7.9 Chronic Toxicity / Carcinogenicity (Tier 3)

Inhalation chronic toxicity and carcinogenicity studies have been conducted for ethylbenzene in rats and mice by the U.S. National Toxicology Program (NTP, 1999). Groups of 50 male and 50 female Fischer 344/N rats and 50 male and female B6C3F1 mice, beginning at 6 weeks of age, were exposed to ethylbenzene by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm (0, 325, 1085, or 3255 mg/m³) for 6 hours/day, 5 days/week for 104 and 103 weeks, respectively.

In the rat study, survival was similar among the female groups but was significantly decreased in the high-dose males compared to the control males (number of males surviving to study termination: 15/50, 14/50, 13/50 and 2/50 at 0, 75, 250 and 750 ppm, respectively). The mean terminal body weights of exposed males and females were 5 to 10% lower than those of the control animals. For chronic (nonneoplastic) effects, the kidney was the major target organ of toxicity in the rat, with renal tubular hyperplasia noted in both males and females at the 750 ppm level only (17/50 vs. 10/50 in controls for males and 8/49 vs. 1/50 in controls for females). The severity of nephropathy was significantly increased relative to the chamber controls in 750 ppm male (3.5 vs. 2.3 in controls) and all exposed female rats (2.3, 1.7, 1.6 and 1.3 for 750, 250, 75 ppm and control groups). The enhanced nephropathy was more severe in males than in females. The incidences of cystic degeneration of the liver was also increased in 750 ppm males (15/50, 12/50, 19/50, 30/49 for chamber control, 75, 250, and 750 ppm); however the biologic significance of this increase is unclear due to the absence of other hepatotoxic changes. Compared to the chamber control group, the incidences of prostate gland inflammation in all exposed groups of males were significantly increased (11/50, 29/50, 22/50, 25/50 for chamber control, 75, 250, and 750 ppm). This inflammatory change consisted of infiltration by predominately mononuclear inflammatory cells with glandular acini and interstitium, increased interstitial fibrosis, and loss of secretory material in affected areas. Also relative to chamber controls, males exposed to 75 or 750 ppm exhibited increased incidences of hyperplasia of the bone marrow characterized by hypercellularity due to the increased numbers of erythroid and myeloid precursor cells (7/49, 16/49, 9/50, 19/50 for chamber control, 75, 250, and 750 ppm). The relationship of these changes to ethylbenzene exposure is uncertain due to the lack of clear concentration-dependent responses. The NOAEL for chronic toxicity was < 75 ppm in females and 250 ppm in males based on kidney pathology.

Ethylbenzene-related tumor findings in the rats were present in the kidney and testis at the highest exposure concentration only. Ethylbenzene administered at 750 ppm was associated with an increase in renal tubule tumors in males after standard evaluation of a single section of each rat's kidney, and in both males and females after evaluation of step-sectioned kidney. The standard histopathological evaluation found a significantly greater incidence in the 750 ppm male rats of renal tubule adenoma (4/50 vs. 0/50 in controls) and adenoma or carcinoma (combined)(7/50 vs. 0/50) than found in the chamber controls. The findings from an extended evaluation (step-section) of the kidney showed a significant increase in the incidences of renal tubule adenoma (17/50 vs. 3/50 in controls for males and 7/49 vs. 0/50 in controls for females); the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 750 ppm males (18/50 vs. 3/50 in controls). In the testis, the incidence of interstitial cell adenoma in 750 ppm males was significantly greater than in the

chamber control group (44/50 vs. 36/50 in controls). The incidence of bilateral testicular adenoma was also significantly increased at 750 ppm (40/50 vs. 27/50 in controls). Although testicular adenomas develop in nearly all aged Fischer rats, in this study ethylbenzene appeared to enhance its development since 92% (22 of 24 rats) of the 750 ppm rats that died between day 400 and day 600 had testicular adenoma, whereas only 33% (3 of 9 rats) of the control that died early had testicular adenoma. The study NOAEL for tumors was 250 ppm in both male and female rats. NTP's cancer conclusion for the study was there was clear evidence of carcinogenicity in male rats due to increased incidences of kidney (renal tubule neoplasms) and testes tumors (testicular adenoma) and some evidence of carcinogenicity in female rats that also showed kidney tumors (renal tubule adenomas), but in a lower incidence and only detected after extended evaluation by step sections.

A further evaluation of the rat kidneys from the NTP study was conducted by Hard (2002) to define the mode of action underlying the tumor development. The reevaluation confirmed the increases in renal tubule incidence in the 750 ppm groups and the increases in the precursor lesion, atypical tubule hyperplasia. The vast majority of the proliferative lesions were of basophilic type and, apart from three carcinomas in the 750 ppm males, either small adenomas or foci of atypical tubule hyperplasia. Also found was a marked exacerbation of chronic progressive nephropathy, an age-related spontaneous disease involving both degenerative and regenerative components, in the 750 ppm males (68% vs. 12% in control males) and a modest exacerbation in 750 females (8% vs. 0% in control females). Almost all of the basophilic tumors occurred in the rats with advanced, usually end-stage, chronic progressive nephropathy, and they were located in areas of parenchyma involved in the chronic progressive nephropathy process. Statistical analysis of the proliferative lesion and the chronic progressive nephropathy data indicated a highly significant correlation between atypical tubule hyperplasia/renal tumor incidence and end-stage chronic progressive nephropathy, and adjusting for end-stage chronic progressive nephropathy removed any statistically significant difference in renal tubule incidence between the ethylbenzene-treated groups and controls. Further, the microscopic examination of renal tubules revealed no evidence of renal tubule injury or increased mitotic activity that would indicate sustained cytotoxicity/cell regeneration as a mode of action of tumor development. Also there was an absence of granular casts and linear papillary mineralization that discounted the possibility of α -2u-globulin nephropathy as the primary underlying basis in male rats, even though subchronic studies had shown a modest accumulation of hyaline droplets in proximal tubules (Stott *et al.*, 2003). Hard's overall conclusion, based on the close association of atypical tubule hyperplasia and renal tumors with chronic progressive nephropathy, was that ethylbenzene-induced exacerbation of chronic progressive nephropathy was the mode of action underlying the development of renal neoplasia, a pathway that is considered to have no relevance for extrapolation to humans.

A short-term exposure study was conducted in rats by Stott *et al.* (2003) to further explore the mode of rat kidney tumorigenesis. Male and female Fischer 344 rats were administered 750 or 75 ppm (3255 or 325 mg/m³) ethylbenzene vapor 6 hours/day, 5 days/week for 1 or 4 weeks. Kidneys were evaluated for changes in organ weights, mixed function oxygenases, glucuronosyl transferase activities, S-phase DNA synthesis, apoptosis, α -2u-globulin deposition, and histopathology. In male rats, exposure to the tumorigenic level of 750 ppm

ethylbenzene vapor resulted in an increase in kidney weight and an initial increase in hyaline droplets and α -2u-globulin in the proximal tubule epithelial cells of the cortex accompanied by an increase in regenerative cell proliferation during the first week of exposure. Early changes were followed by a diminution in α -2u-globulin deposition but continued elevation of S-phase DNA synthesis and histopathologic changes, suggesting chronic progressive nephropathy and a more chronic regenerative cell proliferation, findings consistent with the evaluation by Hard (2002). Both α -2u-globulin nephropathy and chronic progressive nephropathy associated increases in cell turnover are recognized kidney tumor risk factors. The male rats exhibited modest induction of mixed function oxygenase and glucuronosyl transferase activities, primarily following 1-week exposure suggestive of an adaptive response to the metabolic load of ethylbenzene and its metabolites. In contrast to males, female rat kidneys did not display significant histopathological changes nor increased S-phase DNA synthesis and instead a nearly 50% decrease in S-phase synthesis was noted following 1 week exposure with no discernable changes in apoptotic rates. Minimal decreases in all mixed function oxygenase activities were found following 4 weeks of exposure that, combined with S-phase synthesis findings, suggested an alteration or loss of the mixed function oxygenase competent cells in female kidney with increasing exposure period. This change was suggested to possibly serve to accelerate development of chronic progressive nephropathy at a level that does not elicit significant morphological changes or measurable elevations in S-phase DNA synthesis over the time periods examined. Exposure to the nontumorigenic level of 75 ppm for one week caused few changes to the rat kidney.

The incidence of testes Leydig (interstitial) cell tumors in male rats in the NTP study appear to be increased by exposure to 750 ppm ethylbenzene. However, Leydig cell tumors are one of the most frequently occurring endocrine tumors in rodents in chronic toxicity/carcinogenicity studies (Capen, 2001). The incidence of Leydig cell tumors in old rats varies considerably depending upon the strain with the highest incidence found in Fischer rats (the strain used for the ethylbenzene chronic/carcinogenicity study) with the incidence at 2 years of age often approaching 100% (Capen, 2001; Haseman and Elwell, 1996). With such a high background incidence, any additional contribution from chemical exposure is difficult to discern. Also, in contrast to rats, Leydig cell tumors in humans are rare and are different in cellular origin (Haseman and Arnold, 1990; Capen, 2001; Clegg *et al.*, 1997). Hormonal imbalances and a number of clinical substances that cause increases in Leydig cell tumors in rats have not resulted in an increased incidence of Leydig cell neoplasia in man (Capen, 2001). Therefore, Leydig cell tumors, a frequent tumor type in male rats, are not considered an appropriate model for assessing the potential risk to human males of developing this rare testicular tumor (Capen, 2001).

In the mouse study (NTP, 1999) survival was unaffected by exposure to ethylbenzene and no consistent exposure-related effects were observed on body weights. The mean body weights of 750 ppm females were generally lower than the controls from week 24 through week 68 but were similar to the controls from week 72 until the end of the study. For chronic (nonneoplastic) effects, the lung and liver were the principal target organs of toxicity with lesions also present in the thyroid and pituitary glands. In the lung, the incidence of alveolar epithelial metaplasia was significantly elevated in 750 ppm males compared to controls. Nonneoplastic liver lesions in females consisted of eosinophilic foci that were increased in

incidence at 750 ppm. Male mice exhibited increases in the incidences of hepatocyte syncytial alteration at 250 and 750 ppm and increases in the incidences of hypertrophy and necrosis at 750 ppm. Pathology in the thyroid gland consisted of positive trends in the incidence of thyroid follicular cell hyperplasia in males and females with significant increases in incidences relative to controls in both sexes at 750 ppm. The pituitary gland of 250 and 750 ppm females had significantly increased incidences of hyperplasia of the pars distalis. The NOAEL for chronic toxicity was 75 ppm in females and males based on pituitary and liver pathology, respectively.

Ethylbenzene-related tumor findings in the mice were present in the lung and liver of the high exposure concentration group only. Ethylbenzene administered at 750 ppm was associated with an increase in lung tumors in males only and liver tumors in females only. The 750 ppm male mice exhibited a significantly greater incidence of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma (16/50 vs. 5/50 in controls) or carcinoma (combined)(19/50 vs. 7/50 in controls) although these incidences were within the NTP historical control range. The 750 ppm female mice compared to chamber controls had a significantly greater incidence of hepatocellular adenoma (16/50 vs. 6/50) and hepatocellular adenoma or carcinoma (combined) (25/50 vs. 13/50) but were within the NTP historical control range. The increase in eosinophilic foci, a lesion which is judged to be a precursor of hepatocellular adenomas, was a supporting finding in the 750 ppm females. The study NOAEL for tumors was 250 ppm in both male and female mice. NTP's cancer conclusion for the study was there some evidence of carcinogenicity in both sexes; for male mice due to lung tumors (alveolar/bronchiolar neoplasms) and for female mice due to liver tumors (hepatocellular neoplasms).

A further evaluation of the mouse lungs and livers from the NTP study was conducted by Brown (2000). This re-evaluation confirmed the increases in lung and liver tumor incidences in male and female mice, respectively, at 750 ppm. The re-evaluation also revealed decreased eosinophilia of the terminal bronchiolar epithelium of male and female mice of the 750 ppm group. Also, a dose-related increased incidence in multifocal hyperplasia of the bronchiolar epithelium with extension to the peribronchiolar alveolar epithelium was observed in all male treated groups and 250 and 750 ppm females. The author noted that the necrotic hepatocytes in the 750 ppm males were usually that of a coagulation-type necrosis of single or small groups of cells, usually the enlarged, hypertrophied centrilobular hepatocytes. The morphology of this necrosis was histomorphologically different from "apoptosis." Also, the syncytial cells were not the predominant cell type with necrosis.

A short-term exposure study was conducted in mice by Stott *et al.* (2003) to further explore the mode of lung and liver tumorigenesis. Male and female B6C3F1 mice were administered 750 or 75 ppm (3255 or 325 mg/m³) ethylbenzene vapor 6 hours/day, 5 days/week for 1 or 4 weeks. Lungs and livers were evaluated for changes in organ weights, mixed function oxygenases, glucuronosyl transferase activities, S-phase DNA synthesis, apoptosis, and histopathology. Exposure of mice to 750 ppm ethylbenzene vapor caused sustained increases in the levels of cell proliferation in liver as evidenced by increases in mitotic figures and S-phase biosynthesis. A greater incidence of mitotic figures was observed in females than males, consistent with the occurrence of liver tumors in this sex. Levels of S-phase synthesis

were also higher in females than males at every location and exposure period. A regiospecificity of increases in S-phase synthesis in both sexes was apparent with the greatest response in centrilobular hepatocytes. In the mouse lung at 750 ppm, increases in S-phase biosynthesis and loss/renewal of metabolic capacity in bronchiolar epithelium indicated a shift in cell populations, likely in Clara cells. The changes in lungs and liver are suggestive of the formation of a toxic metabolite and regenerative cell proliferation. The nontumorigenic exposure level of 75 ppm resulted in few changes in the lungs and livers of mice.

A couple of chronic oral studies in rats of limited design and detail were performed for ethylbenzene by Maltoni and co-workers (1985, 1997). The first study was comprised of groups of 50 male and 50 female Sprague-Dawley rats that received gavage doses of 0 or 800 mg/kg bwt ethylbenzene (purity, 99.57%) in olive oil solution daily on 4 days/week for 104 weeks. In the second study, groups of 40 male and 40 female Sprague-Dawley rats received 500 mg/kg bwt/day ethylbenzene according to the weekly schedule above while 50 male and 50 female Sprague-Dawley rats were used as control group and received only olive oil. The rats in this study were permitted to live out their natural life span, up to 145 weeks. In these studies, animal survival was affected by treatment with ethylbenzene as indicated as an "intermediate reduction" in animal numbers in both males and females. Reportedly, at 800 mg/kg bwt/day, there was an increase in the incidence of tumors of the nasal cavity (type unspecified)(2% incidence in females versus 0% in controls) and neuroesthesioepitheliomas (6% in males versus 0% in controls) and a borderline increase in tumors of the oral cavity (6% in females versus 2% in controls). The reporting of this study is deficient in details such as numbers of animals with specific tumors, adjustments for survival, historical control data, and statistical analysis; hence an assessment of the study and its results are not possible.

In summary, ethylbenzene has been evaluated in rats and mice for chronic and carcinogenicity effects. The strongest evidence of cancer was kidney tumors found in male rats that inhaled 750 ppm ethylbenzene, a concentration that also significantly reduced the male rats' survival. There was some evidence of kidney tumors in female rats at this concentration that was detected only after extended evaluation. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal neoplasia. Male rats that inhaled 750 ppm ethylbenzene also appeared to have an exacerbation in testicular tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence at 750 ppm ethylbenzene of liver and lung tumors in mice. The incidences of lung tumors in male mice and liver tumors in female mice were greater than those in concurrent control but were within the NTP historical control ranges. Increases in regenerative cell proliferation are postulated to play a key role in the mouse tumor findings. Chronic nonneoplastic toxicity by ethylbenzene principally targeted the kidneys in rats and the liver and lungs of mice at concentrations of 75 ppm and above. Changes of uncertain relevance to ethylbenzene were also apparent in the rat prostate and the mouse thyroid and pituitary glands. No further chronic or carcinogenicity testing of ethylbenzene is recommended.

7.10 Neurotoxicity Screening Battery (Tier 3)

Ethylbenzene has been studied in rodents for both acute and repeated-exposure neurotoxic effects.

A thorough assessment of acute neurobehavioral responses in mice during and following exposure to 2000, 4000, or 8000 ppm (8680, 17360, or 34720 mg/m³) ethylbenzene vapor was performed by Tegeris and Balster (1994). A modification for the mouse using the Functional Observational Battery (FOB) in rats included in the US EPA neurobehavioral toxicity testing guideline (not stated but presumably OPPTS 870.6200) was developed and tested for several alkylbenzenes in this study. The assessment made during exposure was conducted in the last 2 minutes of the inhalation exposure and consisted of the mice being scored on eight measures (posture, arousal, rearing, clonic movements, tonic movements, palpebral closure, gait and gait abnormalities). For the post-exposure assessments, the mice were removed from the exposure chamber within 10 to 15 seconds and evaluated on the complete FOB. The duration of the open field assessment was 2 minutes and a 20 second cutoff was used for the inverted screen test. The entire evaluation required 3 to 4 minutes per mouse. During exposure, ethylbenzene produced dose-dependent (at 2000, 4000, and 8000 ppm) abnormal postures and decrease in arousal and rearing. Statistically significant changes were also present in palpebral closure and gait and gait abnormalities. After exposure, significant changes were present for all or some of the doses on arousal, rearing, ease of removal from chamber, lacrimation, gait and gait abnormalities, mobility, righting reflex, forelimb grip strength, inverted screen test, landing foot splay, approach response, click response, touch response, and tail pinch response. This study is very informative about the nature of the acute neurological response at very high exposure concentrations of ethylbenzene but did not study the effects of lower exposure concentrations or how long effects persist after exposure is terminated.

The acute neurological effects of ethylbenzene in rats were assessed in a limited acute study by Molnar *et al.* (1986). The objective of this study was to examine the pre-narcotic motor behavior of ethylbenzene and also *o*- and *p*-xylene in rats and to compare these effects with the similar hydrocarbons, benzene and toluene. Male CFY rats (8/group) received inhalation exposure of ethylbenzene vapor at concentrations between 100 and 3000 ppm (434 or 13020 mg/m³) for 1, 2, 3, or 4 hours. During-exposure group motility was assessed by recording the number of “touchings” by the moving rats using four electro-mechanical transducers built into metal tubes that were fixed in a perpendicular position within the chamber. Ethylbenzene, similar to the other tested substances, produced a bell-shaped concentration-action curves characteristic of the biphasic effect (*i.e.*, activation at lower and depression at higher concentrations), a finding that was previously described for benzene and toluene. At 400 to 1500 ppm (1736 to 6510 mg/m³), ethylbenzene caused a moderate alteration in motor behavior as indicated by a moderate activation. The minimum narcotic concentration indicated for ethylbenzene in this study was 2180 ppm (9461 mg/m³). Overall, ethylbenzene produced mid-range effects against the other substances tested.

A couple of short-term exposure studies are reported that evaluated neurological effects of ethylbenzene in rats. Andersson *et al.* (1981) exposed rats for 3 days to 2000 ppm (8680 mg/m³) ethylbenzene and found disturbances in neurotransmission in brain areas important to functions in mental and motor control. Junnila and Nasanen (1983) examined behavior patterns of rats exposed to 50 to 600 ppm (217 to 2604 mg/m³) ethylbenzene for 5 days or 7

weeks using an open-field test system. The test parameters recorded were total motor activity, defecation, number of rising on hind legs, jumping, backward steps, circling and frequency and duration of grooming behaviors and complete immobility. Changes appeared in different parameters with different exposure levels, but no dose-response was found; thus these results are difficult to interpret.

A screening assessment of ethylbenzene neurotoxicity was also evaluated in a subchronic oral toxicity study conducted in Wistar rats (Mellert *et al.*, 2004, 2007)[Described in Subchronic Toxicity Section above]. Rats (10/sex/dose) received gavage doses of 0, 75, 250, or 750 mg/kg bwt/day ethylbenzene administered each day as 2 part doses with an interval of about 8 hours. Neurological observations consisted of daily clinical observations, detailed clinical examinations conducted in an open field prior to the start of the administration period and weekly thereafter and a FOB and measurement of motor activity carried out after 13 weeks of treatment. Brain weights were measured on all group animals and brain, sciatic nerve, and spinal cord were routinely preserved and processed for the control and high dose group (750 mg/kg bwt/day) for histopathological examination.

Clinically, post-dose salivation was observed in ≥ 250 mg/kg bwt/day animals that was likely due to local irritation of the test material to the upper digestive tract and hence was not considered a primary neurological effect. The functional neurologic assessment was largely unaffected by ethylbenzene exposure with two exceptions. A decrease in the value of the landing foot-splay test occurred in 750 mg/kg bwt/day males that may have been related to the decrease in body weight in this group. The females in the 750 mg/kg bwt/day group exhibited increased motor activity but the finding was atypical occurring at intervals 3, 6, and 10 (of 12 intervals) suggesting an incidental and not treatment-related finding. Brain weights were not affected by ethylbenzene exposure. Male rats that received ethylbenzene had absolute brain weights similar to the control rats; whereas 750 mg/kg bwt/day males had significantly greater relative (to body weight) brain weights than controls that was correlated with the significantly reduced body weights in these animals. The treated female rats did not show body weight or brain weight changes. There were no treatment related histopathological lesions present in the brain, spinal cord, and sciatic nerve.

For the VCCEP program, a more definitive rat subchronic neurotoxicity study was sponsored by the American Chemistry Council Ethylbenzene Panel and conducted at Charles River Laboratories Argus Division (Barnett, 2006). Sprague-Dawley rats received daily gavage doses of 0, 50, 250, or 500 mg ethylbenzene/kg bwt for 91 consecutive doses. These doses were administered as $\frac{1}{2}$ divided doses 2 times per day approximately 3 hours apart. Sixteen rats/sex/dose were assigned to the control and 500 mg/kg bwt/day groups, and 10 rats/sex/dose were assigned to the 50 and 250 mg/kg bwt/day groups. Detailed clinical examinations were conducted prior to the start of the administration period and weekly thereafter. A FOB and 1-hour motor activity test was conducted prior to exposure and during the 4th, 8th and 13th week of exposure on all rats. These behavioral endpoints were evaluated at the same time of day prior to daily dosing. The detailed clinical examinations and the FOB were conducted by observers who were unaware of the treatment level each rat received and were certified and trained to perform the FOB. The time of testing was balanced across treatment level. At the end of the exposure period, 9 to 11 rats per sex per dose group were perfused *in situ* with neutral buffered 10% formalin and the liver and kidneys weighed. The brains of animals selected for neurohistological examination were weighed. The liver, kidney

and nervous system tissues of all the perfused animals in all dose groups were retained. The kidneys and livers from all perfused animals in the control and 500 mg/kg bwt/day dose group were examined histologically. The eyes, brain, spinal cord, and hindlimb peripheral nerves and muscle from 6 of the rats perfused in the control male dosage group, 7 in the female control dosage group and 6 in the male and female high dosage groups were examined histologically.

There were no treatment-related adverse effects on FOB, motor activity, or neuropathology. There were no statistically significant findings in the vast majority of behavioral measures on the FOB at the different time periods. At 500 mg/kg bwt/day, there were statistically significant findings on the following FOB measures: (1) increased incidence in female rats having normal levels of urination in the open field (week 4); (2) increased incidence in female rats having a startle reaction to acoustic stimuli (week 8); and (3) decreased incidence in male rats having a startle reaction to acoustic stimuli (week 13). These observations were considered spurious statistical findings that are not treatment-related effects because they are normal behaviors occurring with similar incidence in groups during pre-test or in controls during exposure in this study. For example, during week 13, 3 male rats oriented themselves to the acoustic stimuli and 13 male rats startled from the 500 mg/kg bwt/day group compared to all 16 control male rats startled. Both “oriented” and “startle” are normal reactions to acoustic stimulus and the ratio of rats that oriented and startled in the 500 mg/kg bwt/day group is within the range observed for the control group in this study.

There were no statistically significant differences in motor activity levels as measured by the cumulative time spent in movement and number of movements during the 1 hour test session. Using repeated measures analysis, there were no dose-related changes in the pattern of these values during the dosing period or were there dose-related differences between the averages calculated across the different weeks of testing. In addition to the repeated measures analysis, two linear trend analyses (LinDOSE*LinTIME and LinDOSE*QdrTIME) were conducted within the framework of the repeated measures analysis to evaluate the effect of treatment on the within session activity. At week 4, there were no statistically significant differences in the LinDOSE*QdrTIME trend for the number of movements and time spent in movement within each session. However, there was a statistically significant difference in the LinDOSE*LinTIME for the time spent in movement but not the number of movements at 250 and 500 mg/kg bwt/day. The mean time spent in movement within the session was similar for all dosage groups except for the last 10 minutes of the test session, where activity was higher in the 250 and 500 mg/kg bwt/day dosage groups. This is not considered treatment-related because (a) there was no clear dose-related pattern in the averages (the values for the 250 mg/kg bwt/day dosage group generally exceeded those of the 500 mg/kg bwt/day and values for the 50 mg/kg bwt/day dosage group were generally lower than the control values); and (b) there were no statistically significant differences among the dosage groups in the measurements for motor activity after longer exposure durations of 8 and 13 weeks.

The 250 mg/kg bwt/day dosage of ethylbenzene increased the relative weights of the liver and kidneys in male rats, and at 500 mg/kg bwt/day the absolute and relative weights of these organs were increased in both male and female rats. The effects on kidney and liver weight

are considered to be treatment-related effects and are consistent with findings from other subchronic studies conducted with ethylbenzene. There were no treatment-related findings in the histopathology evaluation of the liver and kidney. Absolute brain weights in the male and female rats were unaffected by ethylbenzene at 500 mg/kg bwt/day. The significant increase ($p \leq 0.01$) in the ratio of brain weight to terminal body weight at this highest dose level is attributed to the slight decrease in terminal body weight that occurred in the 500 mg/kg bwt/day dosage group.

At 500 mg/kg bwt/day, there were slight increases in the numbers of male and female rats observed with slight to moderate excess salivation and marginal increases in urine-stained abdominal fur compared to controls. The majority of observations of excess salivation occurred around the time that the daily doses were administered. Therefore, this was likely due to local irritation of the test material to the upper digestive tract and hence is not considered a primary neurological effect. There were incidences of urine-stained abdominal fur in all the dosage groups.

This subchronic neurotoxicity study did not result in effects on motor activity or functional observational battery that were consistent with those observed in the subchronic oral study discussed immediately above (Mellert *et al.*, 2004, 2007). Taken together, these studies indicate that subchronic oral exposure to ethylbenzene does not result in neurotoxic effects at doses that cause treatment-related effects on the kidneys and liver.

Recently specialized investigations have been conducted to evaluate the effects of ethylbenzene on hearing. Several organic solvents, including the aromatic hydrocarbons toluene, xylene, styrene, and ethylbenzene, have been associated with predominately mid-frequency (8 to 20 kHz) range ototoxicity in laboratory animals (Pryor *et al.*, 1983, 1984, 1987; Pryor and Rebert, 1993, Campo *et al.*, 1997). Electrophysiology measurements of the brain and histopathology of the cochlea are the endpoints that have been assessed in the present investigations as indicators of toxicity to the auditory system, although there are currently no standardized testing guidelines available for conducting animal ototoxicity studies. As a recent area of study for ethylbenzene, the available information is presently limited, hence hazard conclusions should be considered preliminary.

In an experiment to evaluate the effect of hearing following exposure of Wag/Rij rats to 800 ppm (3472 mg/m³) ethylbenzene 8 hours/day for 5 days, there was increased auditory thresholds by about 25 dB for startle response at 1 and 4 weeks after the end of the exposure (Cappaert *et al.*, 1999). A shift in the electrocochleography was seen at 8 and 11 weeks after exposure. In a study with lower concentrations of ethylbenzene, 3 to 6 weeks after exposure to 300, 400, or 550 ppm (1302, 1736, or 2387 mg/m³) for 8 hours/day for 5 days, mid-frequency hearing region (8-12 kHz) and auditory thresholds were increased in the 400 and 550 ppm groups. A dose-related outer hair cell loss was found in 2 of the 5 examined regions (11 and 21 kHz) in the cochlea (Cappaert, 2000; Cappaert *et al.*, 2000). In a follow-up study, hearing parameters (as measured by distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAPs)) were altered by noise alone (105 dB) and with noise in combination with ethylbenzene (105 dB + 300 or 400 ppm ethylbenzene). However, the amount of loss after exposure to the combination did not exceed the loss after

noise alone. In this study, ethylbenzene alone (300 or 400 ppm) did not cause significant hearing loss (Cappaert, 2000, Cappaert *et al.*, 2001).

No hearing loss was reported in guinea pigs exposed to 2500 ppm (10850 mg/m³) ethylbenzene 8 hours on the first day, and 6 hours/day for an additional 4 days (Cappaert, 2000; Cappaert *et al.*, 2002). The lack of ototoxicity of ethylbenzene in guinea pigs at external concentrations that produce toxicity in rats was attributed by the authors to lower circulating levels of ethylbenzene.

An oral gavage study was conducted for 21 solvents, including ethylbenzene, looking at histological lesions in the organ of Corti of rats (Gagnaire and Langlais, 2005). Ethylbenzene was administered to one of the groups of 7 to 8 eight-week-old rats by gastric intubation at a dosage of 8.47 mmol/kg bwt (approximately 900 mg/kg bwt) daily for 5 days/week for a 2-week period. The animals were sacrificed 10 days after the end of the treatment period and histopathology examination was conducted on the organ of Corti. The study found ethylbenzene produced almost complete hair cell loss in the 3 rows of outer hair cells in the medium and apical parts of the cochlea. About 50% of the animals also had losses in the basal part of the cochlea. Under the conditions of this study, ethylbenzene was concluded to be among the solvents producing the highest ototoxicity.

Gagnaire *et al.* (2007) have recently completed a subchronic ototoxicity study of ethylbenzene and mixed xylenes vapor in rats. Male Sprague-Dawley rats were exposed to ethylbenzene (200, 400, 600 and 800 ppm; 868, 1736, 2604, and 3472 mg/m³) by inhalation, 6 hours/day, 6 days/week for 13 weeks and sacrificed for morphological investigation 8 weeks after the end of exposure. Brainstem auditory-evoked responses were used to determine auditory thresholds at different frequencies at the end of the 4th, 8th, and 13th weeks of exposure and at the end of the 8th week of recovery. Ethylbenzene produced moderate to severe ototoxicity in rats exposed to the 4 concentrations studied. Increased thresholds were observed at 2, 4, 8 and 16 kHz in rats exposed to 400, 600 and 800 ppm ethylbenzene. Moderate to severe losses of outer hair cells of the organ of Corti occurred in animals exposed to the 4 concentrations studied. Based on the most important cell losses that were found in the 3rd row of the outer hair cells, the authors calculated 371 ppm ethylbenzene as the theoretical concentration causing 50% losses (EC50) in this cell row. Theoretical lowest adverse effect levels (TLAELs) were also calculated from the statistical upper confidence limits of the average losses observed in the controls at 114, 120, and 130 ppm, for 95, 99, and 99.9%, respectively.

In summary, ethylbenzene has been evaluated for acute and subchronic neurotoxicity in laboratory animals and specialized investigations have been conducted to examine auditory effects. Similar to other organic solvents, that at high concentrations exhibit general and non-specific depressant effects (narcosis) on the central nervous system, acute neurological effects can occur with exposure to high concentrations of ethylbenzene. Although not specifically evaluated, these acute nervous system effects that occur at non-lethal concentrations are likely transient effects of acute exposure to ethylbenzene. Alternatively, repeated exposure to concentrations of ethylbenzene that do not produce acute neurological effects but that cause effects to the kidney and liver of laboratory rodents, do not in standard

neurotoxicity studies produce behavioral or morphological effects indicative of specific, persistent, or progressive action of ethylbenzene on the nervous system. Therefore no further standard neurotoxicity testing of ethylbenzene is warranted. Short-term and subchronic exposures to ethylbenzene vapor at concentrations of 200 ppm and greater have been associated with structural and electrophysiological alterations in the auditory system of laboratory animals. This information is recent and an area of continued active research for organic solvents, however, there may be a need for further information on ototoxicity in laboratory animals to clarify this potential toxicity for ethylbenzene.

7.11 Developmental Neurotoxicity (Tier 3)

For the VCCEP program, a rat developmental neurotoxicity study was sponsored by the American Chemistry Council Ethylbenzene Panel and conducted at WIL Research Laboratories, Inc (Stump, 2004a) as a component of the rat 2-generation reproductive toxicity study. The dams for this study consisted of 4 groups of female Crl:CD[®](SD) IGS BR rats (F₁ generation: 25/group) and were exposed to either clean filtered air or vapor atmospheres of the test article, ethylbenzene, at 0, 25, 100, and 500 ppm (0, 108, 434, and 2170 mg/m³) for 6 hours daily, 7 days/week. Inhalation exposure was suspended from gestation day 21 through lactation day 4 and, on lactation days 1 through 4, the dams received the vehicle, corn oil, or test article in the vehicle via oral gavage at dose levels of 0, 26, 90 and 342 mg/kg bwt/day (divided into 3 equal doses, approximately 2 hours apart) at a dose volume of 1 mL/kg bwt/dose. A total of 40 pups/sex/group (2 pups/sex/litter, where possible) from the F₂ generation were selected for evaluation of developmental neurotoxicity. Standard assessments included in the 2-generation component of the study were given to the F₁ females and F₂ pups. Additionally, functional observational battery (FOB) evaluations were performed on the F₁ females on gestation days 6 and 12 and lactation days 10 and 21. Neurobehavioral evaluations conducted on 2 subgroups (each of 20/sex/group) of the F₂ pups included FOB evaluations on postnatal day (PND) 4, 11, 22, 45, and 60, locomotor activity evaluations on PND 13, 17, 21, and 61, acoustic startle response evaluations on PND 20 and 60, and learning and memory evaluations in a Biel water maze task initiated on PND 62 (Subset A) and learning and memory evaluations in a Biel water maze task beginning on PND 26 (Subset B). Following *in situ* perfusion, brain weights and brain dimensions (length and width) were measured for 10 F₂ pups/sex/group on PND 21 (Subset C) and on PND 72 (a portion of animals from Subset A). In addition, a microscopic examination was conducted of the brains (PND 21) or representative portions of the central and peripheral nervous systems (PND 72), including brain morphometric evaluation, of 10 F₂ rats/sex/group from the control and high-exposure groups. F₂ rats used for neurobehavioral testing that were not selected for neuropathology and brain dimension measurements were necropsied on either PND 33 (Subset B) or PND 72 (Subset A).

No adverse ethylbenzene exposure-related survival, clinical observations, findings in FOB assessments, or in macroscopic findings was noted at any exposure level in the F₁ generation maternal animals or in the F₂ offspring. F₂ offspring body weight data and pre-weaning and post-weaning developmental landmarks were unaffected by parental ethylbenzene exposure. There were no statistically-significant or toxicologically-relevant changes in preweaning or PND 60 motor activity parameters, startle parameters assessed on PND 20, or in Biel maze

performance initiated at either PND 26 or 62. Overall mean brain weights, widths, and lengths were not affected at either age of measurement in any of the ethylbenzene-derived offspring. Neurohistopathology and brain area morphometric measurements showed no alterations compared to controls in the offspring derived from the 500 ppm group.

There were some unusual, sometimes statistically significant findings noted in this component of the study, however. These include the apparently precocious appearance of habituation during preweaning activity assessments and very low PND 20 peak startle amplitudes in the control group, as well as the apparent non-dose responsive decrease in male peak startle amplitude on PND 60 for all ethylbenzene-derived groups. There is no evident pattern of parental exposure-related developmental neurotoxicity within one or more CNS domains assessed in offspring in this study. In conjunction with variable timing in appearance of some physical landmarks of development in control animals in both the F₁ and F₂ generations, it seems that the isolated occurrences of these apparent findings are more likely related to unusual variations in the control animals than to parental exposure to ethylbenzene.

The primary finding of possible concern in this component of the study was the apparent decrease in PND 60 peak startle amplitude in all male exposure groups (37% to 49% lower than the concurrent control group). If, indeed, these decreases in peak amplitude were related to parental ethylbenzene exposure, some corroborative evidence of alterations in reactivity in the FOB would be expected to be apparent, at least in males derived from the 500 ppm group. Also, while there are examples of behavior that show sex-specific patterns of response and susceptibility to alteration following developmental neurotoxicant exposure, the basic startle reflex is not one of those. If this finding in all males was indicative of developmental neurotoxicity, it would also be expected to occur in females from all groups, as well as show some evidence of occurrence at PND 20. Therefore, in light of 1) the unusual pre-weaning motor activity pattern, and the remarkable shift from very low values on PND 20 to rather high values on PND 60 in peak amplitude observed in the control animals; 2) the lack of a dose-response in males over a 20-fold parental exposure range; and 3) the lack of findings in other measures of reactivity in either sex, the apparently lower peak response values on PND 60 in the males from all ethylbenzene-derived groups and females from the 500 ppm group were not considered to be related to parental ethylbenzene exposure.

Based on these results, The NOAEL for developmental neurotoxicity was considered to be 500 ppm or 500 ppm/342 mg/kg bwt/day (gavage from gestation day 21 to lactation day 4) of ethylbenzene, the highest exposure level tested in this study.

The potential for ethylbenzene to cause developmental neurotoxicity has been studied using rats from a 2-generation reproductive toxicity study. This study found no adverse effects that could be attributed to ethylbenzene exposure at up to the highest exposure tested of 500 ppm/342 mg/kg bwt/day (gavage from gestation day 21 to lactation day 4). No further developmental neurotoxicity testing for ethylbenzene is needed.

7.12 Human Data

Acute lethality or serious poisoning in humans have rarely been reported in association with ethylbenzene exposure. A worker in an ethylbenzene production facility in Czechoslovakia reportedly died of acute ethylbenzene toxicity after he entered a tank containing “heavy”

concentrations of ethylbenzene vapor (Bardodej and Cirek, 1988). In a study on ethylbenzene metabolism in humans, it was reported that exposures above the occupational limit value of 100 ppm (434 mg/m³) drew complaints of fatigue, sleepiness, headache, and irritation of the eyes and respiratory tract (Bardodej and Bardodejova, 1970). Dizziness was reported in human subjects with 6 minutes of exposure to 2000 ppm (8680 mg/m³) ethylbenzene (Yant *et al.*, 1930).

High concentrations of ethylbenzene vapor are reported to be irritating to human subjects. Irritation of the eyes and respiratory tract were reported following exposure to 100 ppm (434 mg/m³) ethylbenzene vapor (Bardodej and Bardodejova, 1970). At 1000 ppm, subjects experienced eye irritation that rapidly diminished in intensity on continued exposure. A concentration of 2000 ppm (8680 mg/m³) caused immediate severe eye irritation, lacrimation, and irritation of the mucous membranes of the nose. Exposure to a concentration of 5000 ppm (21700 mg/m³) ethylbenzene caused intolerable irritation of the eyes and the mucous membranes of the nose (Yant *et al.*, 1930; Grant, 1986).

The skin sensitization potential of ethylbenzene has been assessed in one limited human study. A patch test using ethylbenzene (10% in petrolatum) on 25 human volunteers was negative for evidence of skin sensitization (Kligman, 1975).

A number of volunteer studies are reported that assessed ethylbenzene disposition in humans. These studies are described in the 'Metabolism and Pharmacokinetics' section of the Hazard Assessment.

There are no reliable human studies or reports on subchronic, chronic, reproductive and development, immune system, nervous system, or genetic toxic effects for ethylbenzene. There have been a number of human studies or reports on health effects associated with hydrocarbon mixtures (*e.g.* paints, gasoline) that contained ethylbenzene as a component, however, these studies can not be used to reliably assess ethylbenzene toxicity. Although not specifically informative on ethylbenzene toxicity, a few of the most commonly referenced human studies are briefly described below.

Angerer and Wulf (1985) studied thirty-five sprayers working with alkyd, phenol and polyester varnishes dissolved mainly in mixed xylenes solvents (including ethylbenzene) for between 2 and 26 years. The sprayers showed on average a higher number of lymphocytes than segmented granulocytes, as well as a slight decrease in the erythrocyte count and hemoglobin level. The level of the alkylbenzenes in the blood and those of their metabolites in the urine were determined, but the data do not permit assessment of the causative agent. The situation is complicated in that the spraymen were exposed to either n-butanol or 1,1,1-trichloroethane in 2 of the 6 workplaces, as well as xylene isomers and toluene. Some of the lacquers also contained leaded pigments.

Triebig *et al.* (1988) conducted a cross-sectional epidemiology study of house painters and neurobehavior effects. The study consisted of 105 house painters and 53 control/non-painter workers. The concentration of work place ethylbenzene was found to be up to 12.9 mg/m³ (3 ppm) and there were also present exposure to ethyl acetate, toluene, butyl acetate, methyl

isobutyl ketone, and xylene. In two of the neurobehavioral tests, change of personality and short-term memory capacity, significant differences were found between painters and controls. In a subgroup of painters with pre-narcotic symptoms at the workplace, the differences were found to be more pronounced. No definitive conclusions on the causative agent for these effects can be drawn from these data.

Spray painting workers exposed to levels of mixed solvents at levels below the German maximum allowable concentration (MAK) values were evaluated for color discrimination effects by Muttray *et al.* (1997). Workers were reportedly exposed to mixed xylenes, ethylbenzene, toluene, propylbenzene, ethyltoluene, methyl ethyl ketone, methyl isobutyl ketone, and perchloroethylene; levels of exposure to individual solvents were not reported, but average combined exposure was about 1/3rd the MAK values. Other chemicals present (e.g., resins, pigments) were not discussed. The workers in this study were found to exhibit a slight increase in the color confusion index in the Lanthony D-15 desaturated test. This small of a change in color discrimination is not likely to be of clinical significance.

Another study reported on nerve conduction effects in ethylbenzene workers (Lu and Zhen, 1989). Minor changes in evoked potential and nerve conduction velocity were found in 22 workers exposed to ethylbenzene concentrations of 0.43 to 17.2 mg/m³ (0.1 to 4 ppm) for 4 to 20 years. These workers also received exposure to styrene (about 1.5 ppm).

A medical surveillance study was conducted on some 200 (exact number not stated) workers involved in the production of ethylbenzene in Czechoslovakia from 1964 to 1985 (Bardoděj and Círek, 1988). Exposure was monitored through mandelic acid concentrations in urine measured twice a year for 10 years. Mandelic acid concentrations in the samples never exceeded 3.25 mmol/L and the mean value was 0.2 to 0.3 mmol/L. According to the authors, a post-shift urine mandelic acid concentration of 6.25 mmol/L was equivalent to an air concentration of 200 mg/m³; therefore the equivalent to air ethylbenzene concentrations for these workers was about 86 and 8.6 mg/m³ (20 and 2 ppm), respectively. None of the workers examined over the last 10 years of the study showed any effects on the levels of hemoglobin, leucocytes or platelets, nor did they have alterations in hematocrit or alanine aminotransferase activity.

Semen quality was evaluated by De Celis *et al.* (2000) in a group of 48 rubber industry workers in Mexico City with exposures for 2 to 24 years to hydrocarbons. The hydrocarbon concentrations determined at the factory were 220.7 to 234 mg/m³ (50 to 54 ppm) ethylbenzene, 31.9 to 47.8 mg/m³ benzene, 189.7 to 212.5 mg/m³ toluene, and 47 to 56.4 mg/m³ xylene. The rubber factory workers were compared to 42 unexposed administrative office workers. The results of this study found that the exposed group had fewer semen with normal characteristics (17%) compared to the unexposed group (76%). Among the increased abnormal semen findings in the exposed workers were alterations in viscosity, liquefaction capacity, sperm count, sperm motility, and the proportion of sperm with normal morphology. Some of these abnormal characteristics correlated with the number of years of exposure to hydrocarbons. Association of these findings to ethylbenzene exposure appears doubtful given the results of the 2-generation rat reproduction study that did not find any abnormal

sperm or fertility effects following exposure to high concentrations of ethylbenzene (Stump *et al.*, 2004a).

Only one study was found that discussed genotoxic effects in humans after inhalation exposure to a mixture of chemicals, including ethylbenzene. Holz *et al.* (1995) determine low-level exposure to ethylbenzene and its effect on peripheral lymphocytes in workers in a styrene production plant. Twenty-five exposed workers were compared with 25 non-exposed control employees working at the same company. The concentration of ethylbenzene for exposed workers determined from active air sampling at four different locations (oven house, production control, storage facility, and distillation area) ranged from 365 to 2,340 mg/m³ (84-539 ppm). Measurements performed at the pump house showed ethylbenzene concentration levels >4,000 mg/m³ (921 ppm) which exceeded the detection limit of the sampling device. Ethylbenzene concentration levels for control workers ranged from 145 to 290 mg/m³ (33-67 ppm). Genotoxic monitoring was performed by nuclease P1-enhanced ³²P-postlabeling of DNA adducts in peripheral blood monocytes, and DNA single strand breaks, sister chromatid exchange, and micronuclei in lymphocytes. The content of kinetochores in the micronuclei was determined by immunofluorescence with specific antibodies from the serum of calcinosis-Raynaud's phenomenon-oesophageal dismobility-sclerodactyltelangiectasia syndrome of scleroderma (CREST) patients. Metabolite concentrations in urine of exposed workers confirmed absorption of the ethylbenzene. No genotoxic effect related to exposure were detected by DNA adduct formation or DNA single strand breaks and sister chromatid exchange. Increase kinetochore positive micronuclei in peripheral lymphocytes were observed in the total exposed group (p=0.007), exposed smokers (p=0.045), and exposed non-smokers (p=0.035); the frequency of total micronuclei in peripheral lymphocytes was unchanged. Results from this study are inconclusive with regard to the genotoxic effects of ethylbenzene, since the workers were exposed to a mixture of styrene, ethylbenzene, benzene, toluene, and xylenes. In addition, the sample size of 25 exposed workers and 25 non-exposed control was very small.

There are no reliable human studies or reports on auditory toxic effects for ethylbenzene. There is some evidence of hearing effects in workers for other aromatic hydrocarbons, although co-exposure to noise complicates interpretation of the human studies.

There are no reliable human epidemiology studies reported that evaluated ethylbenzene exposure and cancer. No reliable epidemiology studies of workers involved in ethylbenzene manufacture have been found nor are there reliable epidemiology studies that examined cancer rates from solvent or gasoline exposure in relation to ethylbenzene concentrations.

Statements on cancer findings were reported in the previously described medical monitoring study of some 200 (exact number not stated) Czechoslovakian ethylbenzene production workers (Bardoděj, and Čírek, 1988). The workers were exposed between 1964 and 1985 and their mean age was 36.6 years and their mean length of employment was 12.2 years. The authors stated that the cancer incidence among chemical workers in the industrial complex (of comparable age and length of employment) not engaged in ethylbenzene production was about three times the national average, whereas in the group of ethylbenzene production workers, no tumors were reported over the previous years. In IARC's review (2000) of this

study, they noted that no precise data was provided to substantiate the author's assertions, there was coexposure to benzene, and the age of the workers and length of the follow-up were not sufficient for a proper evaluation of cancer risk in relation to exposure to ethylbenzene.

A mortality study was conducted among 560 styrene production and polymerization workers employed for at least 5 years on May 1, 1960 at a US plant (Nicholson *et al.*, 1978). Exposures present at the plant were ethylbenzene, benzene, toluene, and styrene. There were 83 deaths observed in the cohort versus 106.4 expected deaths, including 17 cancer deaths (versus 21 expected). Among the deaths, one was from leukemia (0.79 expected) and one was from lymphoma (1.25 expected). A further review of additional death certificates from recent years revealed additional cases of leukemia and lymphoma. IARC (2000) concluded that this study was not useful for evaluation of cancer risk because of deficiencies in the reporting and analysis of the mortality data.

7.13 Hazard Summary

The toxicological effects of ethylbenzene have been thoroughly studied. Ethylbenzene has been evaluated by all the toxicity tests listed in Tier 1, Tier 2, and Tier 3 of the Pilot Announcement and overall this information is of suitable quality to support human health hazard and risk assessments for children and prospective parents. The following provides a summary hazard characterization for ethylbenzene of each of the toxicity endpoints covered by VCCEP.

Acute Toxicity

Animal and human data demonstrate that ethylbenzene has low acute toxicity. High doses are required to produce neurological signs and symptoms and death. High vapor concentrations can be irritating to mucous membranes and liquid can cause irritation to the skin and eyes. Ethylbenzene is not a concern for skin sensitization.

Mutagenicity

Ethylbenzene has been extensively tested for toxicity to genetic material using nearly every available type of genetic toxicity test. Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies. Overall, these study results do not indicate that ethylbenzene is a concern for genotoxicity.

Systemic (Repeated Dose) Toxicity

The repeated exposure (non-cancer) systemic toxicity of ethylbenzene has been evaluated in laboratory animals in subchronic and chronic inhalation studies and a subchronic oral study. Overall, ethylbenzene is a moderate repeated exposure toxicity hazard with consistent targeted effects to the liver and kidney.

In subchronic inhalation studies, the liver and kidney effects were generally limited to increases in organ weights without corresponding histopathological changes that occurred at ≥ 250 ppm ethylbenzene concentrations. The changes in organ weights alone were probably due to metabolic adaptive responses to the high load of ethylbenzene that did not appear to be toxicologically-significant or adverse to the animals on study. However, life-time inhalation exposures of rodents to ethylbenzene did produce pathological lesions in the mouse liver (eosinophilic foci, hepatocyte syncytial alteration, hypertrophy, necrosis) and rat kidney (renal tubular hyperplasia, chronic progressive nephropathy), so the toxicological significance of the early subchronic changes in these organs can not be discounted. Conversely, chronic pathology of significance was not observed in the rat liver or mouse kidney; hence these subchronic organ weight changes do not appear to be precursor subtoxic effects. Pathological changes were also apparent in several other organs in the chronic inhalation studies that were not affected in the subchronic studies. In the mouse, life time ethylbenzene exposure of 750 ppm produced lung pathology (alveolar epithelial metaplasia) and thyroid pathology (thyroid follicular cell hyperplasia) and ≥ 250 ppm ethylbenzene produced hyperplasia of the pituitary gland pars distalis. Rats that received life-time exposures to ethylbenzene also exhibited pathological changes to prostate gland, bone marrow, and liver; however the relationship of these changes to ethylbenzene exposure was deemed uncertain by NTP due to the lack of clear concentration-dependent responses or other correlated toxic changes.

As with inhalation exposure, liver and kidney effects were observed in a subchronic oral study conducted in rats at dosages of ≥ 250 mg/kg bwt ethylbenzene. These effects were more pronounced than were seen in the subchronic inhalation studies as indicated by greater increases in organ weights and secondary changes in clinical chemistry enzymes, minerals, and electrolytes. The subchronic oral study also detected a minimal regenerative anemia and a reduction in prothrombin time, both of questionable significance.

Developmental and Reproductive Toxicity

Ethylbenzene is not a teratogen or reproductive toxicant. At doses that produced maternal effects (≥ 1000 ppm) in laboratory animals, as indicated by adverse clinical signs, reductions in body weight, and increases in organ weights, ethylbenzene was fetotoxic causing decreases in fetal body weights and increases in skeletal variations. No fetotoxicity was present in developmental toxicity studies at 500 ppm or lower ethylbenzene concentrations. Ethylbenzene administered at up to 500 ppm to rats also did not adversely affect reproductive

performance or offspring development over two generations. Estrous cycle length, pre-coital intervals, male and female mating and fertility indices, gestation length, spermatogenic endpoints, and reproductive organ weights were unaffected by exposure to 500 ppm ethylbenzene. No adverse effects were also seen on ovarian follicle counts, the pup litter parameters of pup sex ratios, live litter sizes, number of dead pups, viability indices, pup body weights, and the general physical condition of the pups. The pre-weaning developmental landmarks pinnal detachment, hair growth, incisor eruption, and eye opening, and the post-weaning developmental landmarks of balanopreputial separation and vaginal patency were unaffected by 500 ppm ethylbenzene exposure. In the pilot study to the reproductive toxicity study, dose-related decreases in offspring preweaning and postweaning body weights, as well as offspring survival immediately following weaning and initiation of exposure occurred at ≥ 500 ppm ethylbenzene; however these effects were not reproduced at 500 ppm in the definitive reproductive toxicity study.

Immunotoxicity

There is no evidence that ethylbenzene is harmful to the immune system. A screening-level immunotoxicity study was conducted for ethylbenzene in rats and this study found no evidence of adverse effects on the functional ability of the humoral component of the immune system (as measured by splenic IgM antibody forming cell response to the T-dependent antigen, sheep erythrocytes) for up to 500 ppm ethylbenzene vapor administered for 28 days. Additionally, in the several subchronic and chronic toxicity studies that have been performed for ethylbenzene, there were no reported weight changes or microscopic lesions affecting immune system organs or tissues.

Metabolism and Pharmacokinetics

The disposition of ethylbenzene in animals and humans has been well characterized. Ethylbenzene is well absorbed from the skin, lungs and gastrointestinal tract, rapidly distributed in the body, metabolized primarily via hydroxylation of the two carbons of the side-chain and then further oxidized to a range of metabolites that are excreted principally in the urine. Differences are apparent between animal species and sexes in aspects of metabolism and overall clearance of ethylbenzene.

Carcinogenicity

Ethylbenzene is carcinogenic in animals following lifetime exposures to high vapor concentrations. The strongest evidence of cancer was kidney tumors found in male rats that inhaled 750 ppm ethylbenzene, a concentration that also significantly reduced the male rats' survival. There was some evidence of kidney tumors in female rats at this concentration that was detected only after extended evaluation. Exacerbation by ethylbenzene of chronic

progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal cancer. Male rats that inhaled 750 ppm ethylbenzene also appeared to have an exacerbation in testicular tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence at 750 ppm ethylbenzene of liver and lung tumors in mice. The incidences of lung tumors in male mice and liver tumors in female mice were greater than those in concurrent control but were within the NTP historical control ranges. Increases in regenerative cell proliferation are postulated to play a key role in the mouse tumor findings.

Neurotoxicity

Consistent with the known effects of organic solvents which cause a general and non-specific depression of the nervous system, acute exposure to high concentrations of ethylbenzene can induce acute neurological effects. Repeated exposure to ethylbenzene at concentrations up to 500 ppm vapor or oral dosages of up to 500 mg/kg bwt/day, however, do not produce any behavioral or morphological effects in standard neurotoxicity studies that are indicative of a specific, persistent, or progressive action on the nervous system. Specialized investigations of ethylbenzene effects on hearing do indicate ethylbenzene can cause ototoxicity. Ototoxicity has been reported for other aromatic hydrocarbons and in a recent 13-week study in rats that found alterations in brainstem auditory evoked responses and outer hair cell morphology in rats at concentrations of 200 ppm and greater ethylbenzene. Therefore, hearing effects may be a concern for ethylbenzene.

Developmental Neurotoxicity

Ethylbenzene is not (selectively) toxic to the developing nervous system. Developmental neurotoxicity was evaluated in rats as a component of the 2-generation reproductive toxicity study for ethylbenzene. This study found no exposure-related effects on functional observational battery assessments, locomotor activity, acoustic startle responses, learning and memory evaluations in a Biel water maze task, and neurohistopathology and brain area morphometric measurements at up to 500 ppm ethylbenzene.

7.14 Robust Summaries of Toxicology Studies

The OECD SIDS Dossier and SIAR and IUCLID (Appendix A) contain summaries of most of the key toxicological studies of ethylbenzene. Expanded robust summaries for 35 studies are found in Appendix O.

SECTION 8. TOXICITY REFERENCE VALUE DERIVATION

8.1 Introduction

Ethylbenzene has been extensively tested for toxicity (see Section 7, Hazard Evaluation). An evaluation of the cancer endpoints, potential modes of action, and cancer potency were considered separately from the noncancer endpoints (see Section 8.3, Cancer Dose Response Assessment for Ethylbenzene). Existing noncancer reference concentration (RfC) and reference dose (RfD) values from U.S. EPA's Integrated Risk Information System (IRIS) were derived in 1991 and 1988, respectively. Since that time, many additional studies pertaining to the toxicity, toxicokinetics, and potential mode of action (MOA) of ethylbenzene toxicity have been conducted. Proposed reference values that reflect the current state of knowledge regarding the noncancer effects were derived as described below.

8.2 Noncancer Toxicity Reference Values (RfC and RfD) for Ethylbenzene

8.2.1 Outline of Key Decisions for Noncancer Reference Value Derivation

Reference values for ethylbenzene were derived using the following equation:

$$RfV = \frac{BMD}{UFA * UFH * UFS * UFL * UFD}$$

Where,

- RfV = Reference value.
- BMD = Benchmark dose.
- UFL = Uncertainty factor for effect level extrapolation. Uncertainty results when an "effect" level (LOAEL) rather than a "no effect" level (NOAEL) is used as the point of departure. Although not typically applied to a BMD value, the UFL has been used in this assessment to account for the severity of the endpoint when warranted.
- UFS = Uncertainty factor for extrapolation from a subchronic effect to a potential chronic effect. When a subchronic study is used to assess potential hazards of chronic exposure, there is uncertainty as to whether additional or more severe effects may have been observed if the study had been of a longer duration.
- UFA = Uncertainty factor for animal to human extrapolation. Extrapolation from animal data rather than human data introduces uncertainty into the assessment. This uncertainty is accounted for by the introduction of the adjustment factor UFA. This uncertainty factor is understood to represent interspecies differences in chemical disposition (pharmacokinetics) and response to the delivered dose (pharmacodynamics).

- UFH = Uncertainty factor for sensitive human subpopulations. The unknown variation in susceptibility among the human population is a source of uncertainty in the risk assessment. This variation is accounted for by including the adjustment factor UFH. Similar to UFA, UFH is understood to represent intraspecies differences in pharmacokinetics and pharmacodynamics.
- UFD = Uncertainty factor for database sufficiency. The lack of an extensive testing database (e.g., two-generation reproductive toxicity, chronic studies, studies in multiple species) may be a source of uncertainty in risk assessments.

The composite UF is determined by multiplying the uncertainty factors for the defined criteria.

Proposed reference values were derived by following the process outlined below.

(1) The RfC/RfD derivation for ethylbenzene involved evaluating multiple potential key studies and endpoints (see Section 7, Hazard Evaluation, and Appendix O Robust Summaries, for study details). Proposed MOA for endpoints and their relevance to humans were evaluated and corresponding internal dose metrics selected. MOA were considered relevant to humans unless otherwise specified below. Candidate studies/effects were considered suitable for RfC/RfD derivation if the quality of the study was adequate and the endpoint was relevant and important to humans. Studies with high NOAELs (500 ppm or higher) were eliminated from consideration due to the existence of studies with substantially lower NOAELs/LOAELs.

(2) For the candidate studies, internal dose estimates corresponding to all tested doses were calculated using the PBPK models. Calculated internal doses were used for dose-response analysis using U.S. EPA's Benchmark Dose Software (BMDS). Based on evaluation of goodness of fit (determined by statistical evaluation and visual inspection), a point of departure (e.g., LED10) was identified.

(3) Uncertainty factors were applied to the point of departure, and the human PBPK model was used for interspecies extrapolation to derive RfCs/RfDs for the given endpoint, assuming continuous, constant exposure/ingestion.

(4) In the case of multiple endpoints, the lowest reference value for a given route of exposure is the proposed reference value. Consideration has been given to deriving an RfD based on findings in inhalation studies due to the more extensive database for this route of exposure.

8.2.2 Noncancer RfC Derivation

Several studies were considered as a potential basis for the RfC. These select studies are summarized briefly in **Table 8-1**.

Table 8-1. Studies Considered for Ethylbenzene RfC Derivation

Endpoint	Species	Reference	NOAEL (ppm)	LOAEL (ppm)
Kidney (incidence), mortality	Rat	NTP (1999)	250	750
Kidney (severity)	Rat	NTP (1999)	Not determined	75
Fertility and reproduction	Rat	Faber <i>et al.</i> (2006)	500	Not applicable
Developmental toxicity	Rabbit	Andrew <i>et al.</i> (1981), Hardin <i>et al.</i> (1981)	100 (EPA), 1000 (American Chemistry Council)	1000 (EPA) Not applicable (American Chemistry Council)
Developmental toxicity	Rat	Andrew <i>et al.</i> (1981), Hardin <i>et al.</i> (1981)	100	1000
Developmental toxicity	Rat	Saillenfait <i>et al.</i> (2003)	500	1000
Ototoxicity	Rat	Gagnaire <i>et al.</i> (2007)	200 (audiometric threshold) Not determined (outer hair cell loss)	400 (audiometric threshold) 200 (outer hair cell loss, LOEL)
Liver, pituitary	Mouse	NTP (1999)	75	250

8.2.2.1 NTP (1999), Rat Chronic Toxicity Study

Male and female F344 rats were exposed to 75, 250, or 750 ppm ethylbenzene for 6 hours/day, 5 days/week for two years. The key noncancer findings of this study were increased incidence of renal tubular hyperplasia in male and female rats at 750 ppm, increased severity of nephropathy in all groups of dosed females and high-dose males, and decreased survival of male rats at 750 ppm (**NOAEL of 250 ppm**) (NTP, 1999). The renal tubular hyperplasia was related to chronic progressive nephropathy (CPN), a rat disease with no human correlate (Hard, 2002). Furthermore, the decreased survival of male rats was considered likely to be related to these same renal effects (NTP, 1999). Thus none of the noncancer effects observed in rats in this study are likely to be relevant to human risk. The study can thus be interpreted as supporting a **NOAEL of 750 ppm** for adverse effects relevant to human health. Because other studies showing effects potentially relevant to humans have much lower NOAELs/LOAELs, this study was not considered to be a candidate for RfC derivation.

8.2.2.2 Faber *et al.* (2006), Rat Two-Generation Study

This two-generation study, which used Sprague-Dawley rats, indicated a **NOAEL of 500 ppm** (the highest tested dose) for parental and neonatal toxicity as well as for toxicity to reproduction. Because other studies in rats show lower NOAELs, this endpoint was not further considered for RfC derivation.

8.2.2.3 Andrew *et al.* (1981), Hardin *et al.* (1981), and Saillenfait *et al.* (2003); Rat and Rabbit Developmental Toxicity Studies

The existing IRIS RfC for ethylbenzene is 1 mg/m³ (0.2 ppm) (IRIS, 1991), based on a NOAEL of 100 ppm and LOAEL 1000 ppm for developmental effects in rats and rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981). We deemed the effects in rabbits at 1000 ppm (4.34 mg/L, a concentration that exceeds the limit dose of 2 mg/L) to be equivocal, and that this study is indicative of a **NOAEL of 1000 ppm for developmental effects in rabbits**, as described in the robust summaries. Because other studies show much lower NOAELs, this endpoint was not further considered for RfC derivation.

The skeletal variants observed in Wistar rats at 1000 ppm (again, exceeding the limit dose) by Andrew *et al.* (1981) and Hardin *et al.*, (1981) are considered marginally adverse. The lack of similar findings in Sprague-Dawley rats concentrations of 100, 500 and 1000 ppm (Saillenfait *et al.*, 2003), in a study apparently conducted in accord with current U.S. EPA and OECD standards, supports the contention that the finding in the earlier studies is of questionable relevance, although strain differences could also play a role. The Saillenfait *et al.* (2003) study identified a developmental toxicity **NOAEL of 500 ppm** and LOAEL of 1000 ppm in rats for decreased fetal bodyweight. Because other studies showing effects potentially relevant to humans have much lower NOAELs/LOAELs, this study was not considered to be a candidate for RfC derivation.

8.2.2.4 Gagnaire *et al.* (2007), Rat Subchronic Ototoxicity Study

Key Findings

Male Sprague-Dawley rats were exposed to 200, 400, 600, or 800 ppm ethylbenzene for 6 hours/day, 6 days/week for 13 weeks. The key finding was **ototoxicity**. Evidence of ototoxicity included increases in the audiometric threshold and outer hair cell loss. Outer hair cell loss was the more sensitive indicator of damage relevant to hearing loss, with “significant” increases in the losses in the third row of outer hair cells (OHC3) at **200 ppm (LOEL)**. It is not clear that this effect can be considered an “adverse”, subchronic effect due to the lack of increases in audiometric threshold, but OHC loss may be relevant to chronic, age related hearing loss. Audiometric thresholds were measured at 2, 4, 8, or 16 kHz after 0, 4, 8, or 13 weeks of exposure with no recovery period or 13 weeks of exposure with an 8-week recovery period. Threshold increases were greatest at 16 kHz for animals without a recovery period. The **NOAEL for increases in audiometric threshold was 200 ppm**, with a **LOAEL of 400 ppm** (Gagnaire *et al.*, 2007).

Proposed MOA and Internal Dose Metric for Ototoxicity

The MOA for ototoxicity in rats is proposed to be related to parent compound concentrations in the organ of Corti, approximated as concentrations in richly perfused tissue. The ototoxic effects of ethylbenzene are likely related to the irreversible loss of outer hair cells (OHC) in the organ of Corti (a region of the cochlea) (Cappaert *et al.*, 1999, 2000, 2001, 2002; Gagnaire and Langlais, 2005; Gagnaire *et al.*, 2007). No ototoxic effects were observed in Wistar rats consuming 5000 mg/L phenylglyoxylic acid, a major ethylbenzene metabolite, in drinking water for 3 months (~293 mg/kg/day) (Ladefoged *et al.*, 1998). The lack of ototoxicity of ethylbenzene in guinea pigs at external concentrations that produce toxicity in rats was attributed to lower circulating levels of ethylbenzene by Cappaert *et al.* (2002). Studies with uninduced and phenobarbital-induced rats exposed to toluene (a compound similar in structure to ethylbenzene) indicate that metabolites are not responsible for toluene-induced ototoxicity (Pryor *et al.*, 1991).

The proposed internal dose metric is the AUC of ethylbenzene in richly perfused tissues (AUCR). The selected point of departure was the lower confidence limit on the effective dose predicted to cause a loss of 1.05% of OHC3, determined using BMDS. This level of loss represents the 95% UCL on OHC3 losses in control rats (Gagnaire *et al.*, 2007). Individual animal data on OHC3 loss were kindly provided by Dr. Francois Gagnaire, to clarify the data presented in Figure 4A of Gagnaire *et al.* (2007) (personal communication). This point of departure is highly conservative as OHC losses of up to 50% in the apical region of the cochlea do not cause measurable hearing loss (Prosen *et al.*, 1990), consistent with the finding that the NOAEL for audiometric threshold changes for ethylbenzene (200 ppm) produced OHC3 losses of $3.67 \pm 4.24\%$ while OHC3 losses were $67.12 \pm 12.26\%$ at the audiometric threshold change LOAEL of 400 ppm (Gagnaire *et al.*, 2007).

Proposed Uncertainty Factors for Ototoxicity

Proposed uncertainty factors for the assessment of ototoxicity are summarized below.

- A UFL = 1 is proposed because a conservative point of departure was selected and the value is derived using benchmark dose modeling.
- A UFS of 1 was previously proposed for OHC3 losses (American Chemistry Council, 2006). A value of 3 is used in this risk assessment. No measured effects on hearing were noted at 200 ppm in the subchronic study. The more sensitive endpoint of OHC3 loss is not indicative of a subchronic adverse effect, but rather a potential susceptibility to chronic, age-related effects (Gagnaire *et al.*, 2007). Furthermore, prolonged exposure (up to 19 months) to toluene did not decrease the NOEL for OHC loss as compared to shorter exposures (1 month or less) (Johnson and Nylen, 1995). As noted in the VCCEP meeting report (2007), “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.” For the purposes of this risk assessment, the conservative value of 3 will be used, though a value of 1 could be considered more appropriate.

- A **UFA** of 3 is proposed. The default UFA of 10 can be divided into pharmacokinetic and pharmacodynamic components each equal to a factor of ~3. Validated physiologically based-pharmacokinetic (PBPK) models appropriate for the interspecies extrapolation of tissue levels of ethylbenzene from rats to humans are available (see Appendix P, Evaluation and Extension of PBPK Models of Ethylbenzene for use in VCCEP Assessment), so the pharmacokinetic component of UFA can be set equal to 1. A factor of 3 is recommended for the pharmacodynamic portion of UFA. This approach is consistent with U.S. EPA RfC guidelines (U.S. EPA, 1994).
- A **UFH** of 10, the default value is proposed. Sensitivity analyses for AUCR predictions in humans indicate that this value of UFH is adequate for protection of children.
- A **UFD** = 1 is proposed because ethylbenzene has been extensively tested by the inhalation route, including chronic studies in both mice and rats (NTP, 1999) and a two-generation reproduction and developmental toxicity test (Faber *et al.*, 2006).

Therefore a composite UF of 100 (1x3x3x10x1) is recommended for a potential ethylbenzene RfC based on rat ototoxicity (OHC3 loss).

8.2.2.5 NTP (1999), Mouse Chronic Toxicity Study

Key Findings

The NTP (1999) chronic cancer bioassay in mice also identified noncancer effects in mice that should be considered for RfC derivation. In this study, male and female B6C3F1 mice were exposed to 75, 250, or 750 ppm ethylbenzene 6 hours/day, 5 days/week for two years. The key findings were (1) **pituitary hyperplasia** in female mice (**NOAEL 75 ppm**, LOAEL 250) and (2) **liver effects** in male mice (liver syncytial alteration **NOAEL 75 ppm**, LOAEL 250), with additional significant liver effects at 750 ppm (male and female mice).

Proposed MOA and Internal Dose Metrics

Proposed MOA and Internal Dose Metrics for Liver Effects

The MOA for **liver syncytial alteration** in male mice is likely similar to the liver cancer MOA (discussed in Section 8.3). If related to a phenobarbital-like MOA, the relevance to the human will need to be considered. Although human experience argues against the human relevance of phenobarbital-induced liver tumors (Holsapple *et al.*, 2006), this

argument does not necessarily hold true for noncancer effects in the liver. Phenobarbital produces hepatic induction in mammalian species including humans, but while liver tumors are observed in laboratory rodents, they are not observed in humans. It is unclear precisely at what point in the continuum of severity for hepatic response between induction and tumor response that laboratory rodents and humans begin to differ. Under this MOA, the proposed dose metric is AUC of ethylbenzene in the liver. Alternatively if the liver effects are related to the formation of reactive metabolites, the proposed dose metric is amount metabolized in the liver/liver weight, and these effects are likely relevant to humans.

Proposed MOA and Internal Dose Metrics for Pituitary Hyperplasia

The MOA for **pituitary hyperplasia** in female mice is potentially related to dopamine depletion by ethylbenzene metabolites. Dopamine depletion in brain has been observed in rabbits exposed systemically to ethylbenzene or its metabolites mandelic and phenylglyoxylic acid (Mutti *et al.*, 1988). Dopamine has an inhibitory effect on lactotroph proliferation in the mouse pituitary gland (Saiardi *et al.*, 1997). Sexual dimorphism in pituitary prolactin levels, providing an additional proliferative signal, would account for the male/female differences in this endpoint in ethylbenzene-exposed mice (females are affected, while males are not).

Ethylbenzene metabolites are likely to be hydrophilic rather than lipophilic, and thus distributed relatively evenly throughout the body. Ethylbenzene is not known to be metabolized in the brain. Thus the proposed internal dose metric is total amount of ethylbenzene metabolized/body weight. The dose response (equivalent incidence at 250 and 750 ppm) is also suggestive of approaching saturation; metabolism would increase somewhat between 250 and 750, but blood concentration would increase more dramatically.

Mouse PBPK Model Internal Dose Estimates

Because of uncertainty in the parameterization of the mouse PBPK model (Appendix P), internal doses were calculated for two parameters sets. For one set, extrahepatic metabolism was assumed to take place only in the lung. For the other parameter set, extrahepatic metabolism in both the lung and richly perfused tissues compartment (RPT) was assumed, with the maximal rate in the lung estimated from *in vitro* data (Saghir and Rick, 2005).

Proposed Uncertainty Factors

- A UFL of 1 is appropriate because the key study identified a NOAEL for increases in pituitary hyperplasia (females) or liver effects (males).
- A UFS of 1 is appropriate because a chronic study was used.

- A **UFA** of 3 is proposed. As noted above (Section 8.2.2.4), the default UFA of 10 can be divided into pharmacokinetic and pharmacodynamic components each equal to a factor of ~3. Validated PBPK models appropriate for the interspecies extrapolation of total metabolism, liver metabolism, or liver concentration of ethylbenzene in mice and humans are available (see Appendix P), so the pharmacokinetic component of UFA can be set equal to 1. We recommend retention of the full factor of 3 for the pharmacodynamic portion of UFA. This approach is consistent with U.S. EPA RfC guidelines (U.S. EPA, 1994).
- A **UFH** of 10, the default value is proposed. Sensitivity analyses for humans indicate that this value of UFH is likely to provide adequate for protection of children (Appendix P).
- An uncertainty factor for database sufficiency (**UFD**) = 1 is proposed because ethylbenzene has been extensively tested by the inhalation route, as noted above (Section 8.2.2.4).

Therefore a composite UF of 30 (1x1x3x10x1) is recommended for the ethylbenzene RfC.

8.2.2.6 Study and Endpoint-Specific RfC Derivations

RfC for Ototoxicity

Ototoxicity BMDS Analysis

The only acceptable fit obtained for the selected dose metric was found for the Hill model (Akaike's Information Criterion [AIC] = 160, p = 0.642). Details are provided in Appendix Q. The saturation of the response indicated by the Hill model is consistent with the data, which showed similar increases in OHC3 loss at 600 and 800 ppm (85.6 ± 7.7 and $90.8 \pm 7.4\%$, respectively) (Gagnaire *et al.*, 2007). The 95% upper confidence limit on the dose producing hair loss exceeding those in control rats (1.05%), LED0105 was 272.8 mg-hr ethylbenzene/L of richly perfused tissue (RPT)/week, which is equivalent to an average tissue concentration of 1.6 mg/L.

Ototoxicity RfC Derivation

Dividing the LED0105 (272.8 mg-hr ethylbenzene/L of RPT/week) by the composite UF of 100 yielded a target human internal dose (AUCR) of 2.73 mg-hr ethylbenzene/L of RPT/week. Weekly AUCR was calculated as the difference between the AUCR for 504 hours (3 weeks) and AUCR for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfC for ototoxicity** was 0.33 ppm, which rounds to **0.3 ppm**.

RfC for Liver Effects

BMDS Analysis of Liver Effects

The selected benchmark response was the 95% lower confidence limit on the dose producing a 10 percent increase in liver effects above background incidence (LED10). A 10% increase in extra risk is considered to be the default benchmark response rate (USEPA, 2005e), and is considered appropriate for this data set.

Using AUCL as the dose metric introduces considerable nonlinearity into the dose-response relationship since the highest concentration is above metabolic saturation, and as a result the only acceptable fits used log-transformed dose metrics. Because it is expected that PBPK modeling should resolve nonlinearity when an appropriate internal dose measure is used, this observation offers empirical support against the use of parent chemical in the target tissue in the dose response assessment. Since the purpose of using an internal dose, such as AUCL, rather than external dose is to provide biological relevance, it does not make sense to use log-transformed internal doses in the dose response. The need to perform log-transformation of AUCL to achieve acceptable dose-response fits indicates that AUCL is not a relevant dose metric for the observed liver effects.

Using AM/VL as the dose metric, log-transformed dose metrics were excluded based on the rationale provided above. Of the remaining models, the best fit was provided by the Gamma, Multistage, Q-Linear, and Weibull models (AIC = 154, $p = 0.109$), producing an LED10 = 3,535 mg ethylbenzene metabolized/kg liver/week for the PBPK model parameter set with no RPT metabolism, AIC = 151 for the Gamma, Multistage, Q-Linear, and Weibull models, $p = 0.298$, and LED10 = 3,875 mg metabolize/kg liver per week for the PBPK model parameter set including RPT metabolism. Details are provided in Appendix Q. On the basis of conservatism, the lower LED10 was used for subsequent calculations.

RfC Derivation for Liver Effects

Dividing the LED10 (3,535 mg ethylbenzene metabolized/kg liver/ week) by the composite UF of 30 yielded a target human internal dose (AM/VL) of 118 mg ethylbenzene metabolized/kg liver/week. Weekly AM/VL was calculated as the difference between the AM/VL for 504 hours (3 weeks) and AM/VL for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfC for liver effects** was 0.84 ppm, which rounds to **0.8 ppm**.

RfC for Pituitary Effects

BMDS Analysis of Pituitary Hyperplasia

The best fit was provided by the Gamma, Multistage, Q-Linear, and Weibull models (AIC = 244, $p = 0.121$), producing an LED10 = 556 mg ethylbenzene metabolized/kg bwt/week. Details are provided in Appendix Q.

RfC Derivation for Pituitary Hyperplasia

Dividing the LED10 (556 mg ethylbenzene metabolized/kg bwt/week) by the composite UF of 30 yielded a target human internal dose (AM/BW) of 18.5 mg ethylbenzene metabolized/kg bwt/week (2.6 mg/kg bwt/day). Weekly AM/BW was calculated as the difference between the AM/BW for 504 hours (3 weeks) and AM/BW for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfC for pituitary hyperplasia** was 5.1 ppm, which rounds to **5 ppm**.

8.2.2.7 Proposed RfC

The potential RfC derived on the basis of ototoxic effects of ethylbenzene observed in a subchronic study in rats was 0.3 ppm. The potential RfC derived on the basis of liver effects of ethylbenzene observed in a chronic study in mice was 0.8 ppm. The potential RfC derived on the basis of pituitary hyperplasia observed in a chronic ethylbenzene study in mice was 5 ppm. For the sake of conservatism, the RfC of 0.3 ppm based upon ototoxicity is adopted for ethylbenzene.

8.2.2.8 Discussion of Proposed RfC

If benchmark dose analysis and PBPK modeling were not used, the default ototoxicity RfC would likely be derived using one of three approaches:

1. Ototoxicity NOAEL = 200 ppm (audiometric threshold change)
Duration adjustment = (6 hours/day × 6 days/week for intermittent exposure)/(168 hours/week for continuous exposure)
Uncertainty factors: same as above (Section 8.2.2.5), except UFA = 10, and UFS = 10, resulting in a composite UF of 1000

$$\text{Default ototoxicity RfC (approach 1)} = 200 \times (36/168)/1000 = 0.04 \text{ ppm}$$

Alternatively, the ototoxicity RfC could be derived based on OHC3 loss as a precursor to chronic effects using the LOEL.

2. Ototoxicity LOEL = 200 ppm (OHC3 loss)
Duration adjustment = (6 hours/day × 6 days/week for intermittent exposure)/(168 hours/week for continuous exposure)
Uncertainty factors: same as above (Section 8.2.2.5), except UFA = 10, UFL = 3 resulting in a composite UF of 300.

$$\text{Default ototoxicity RfC (approach 2)} = 200 \times (36/168)/300 = 0.1 \text{ ppm}$$

Lastly, the ototoxicity RfC could be derived based on OHC3 loss as a precursor to chronic effects using the Theoretical Lowest Adverse Effect Level (95% UCL) or TLAEL derived by Gagnaire *et al.* (2007).

- Ototoxicity TLAEL= 114 ppm (OHC3 loss)
Duration adjustment = (6 hours/day × 6 days/week for intermittent exposure)/(168 hours/week for continuous exposure)
Uncertainty factors: same as above (Section 8.2.2.5), except UFA = 10, resulting in a composite UF of 300.

$$\text{Ototoxicity RfC (approach 3)} = 114 \times (36/168)/300 = 0.08 \text{ ppm}$$

Likewise, the default liver and pituitary toxicity RfCs would be derived as follows:

Liver and Pituitary Toxicity NOAEL = 75 ppm
Duration adjustment = (6 hours/day × 5 days/week for intermittent exposure)/(168 hours/week for continuous exposure)
Uncertainty factors: same as above, except UFA = 10, resulting in a composite UF of 100

$$\text{Default liver RfC} = 75 \times (30/168)/100 = 0.1 \text{ ppm}$$

The existing IRIS RfC for ethylbenzene is 1 mg/m³ (0.2 ppm) (U.S. EPA, 1991), based on a NOAEL of 434 mg/m³ (100 ppm) and LOAEL of 4340 mg/m³ (1000 ppm) for developmental effects in rats and rabbits (Andrew *et al.*, 1981; Hardin *et al.*, 1981). We deemed the effects at 1000 ppm in rabbits to be equivocal, and that this study is indicative of a NOAEL of 1000 ppm for developmental effects in rabbits, as described in the robust summaries. The NOAEL of 100 ppm (with a LOAEL of 1000 ppm) for developmental effects in rats (Andrew *et al.*, 1981; Hardin *et al.*, 1981) is superseded by the NOAEL of 500 ppm of Saillenfait *et al.* (2003). Additionally, the existing IRIS RfC was calculated by applying a total UF of 300, which included a database factor of 10 for the lack of a multigeneration reproductive study and the lack of chronic studies. U.S. EPA assigned confidence ratings of “low” to the study, the database, and the RfC. Both of the gaps cited for the ethylbenzene toxicity database have been filled since the U.S. EPA IRIS assessment.

Overall, the confidence in the proposed RfC is medium-to-high. The toxicity-testing database is extensive, indicating that it is unlikely that there are toxicologically important effects of ethylbenzene that have not been identified. The confidence in the PBPK-derived internal dosimetry estimates for the range used in liver dose response is high. Because ototoxicity has not, to the best of our knowledge, previously been used as the basis of an RfC, precedents for the selection of an effect level are lacking. We have chosen a conservative effect level and uncertainty factors for this endpoint and derived a potential RfC for ototoxicity that is slightly lower than derived for the critical endpoint, liver effects.

The proposed RfC (0.3 ppm) is slightly higher than the existing RfC (0.2 ppm), due to use of a more sensitive, previously untested endpoint and a smaller uncertainty factor, but can be assigned greater confidence. The differences are due to a different interpretation of the rabbit developmental data, the conduct of an additional rat developmental toxicity

study which increased the rat developmental toxicity NOAEL, the selection of key studies that were not available when the existing RfC was derived, use of benchmark dose modeling (instead of the NOAEL) to identify the point of departure, differing values for components of the composite UF (as compared to the previous key studies), the existence of studies for additional endpoints and durations (i.e., multigeneration reproduction and chronic studies) and use of PBPK modeling for interspecies extrapolation (instead of using the default UFA).

8.2.3 Noncancer RfD Derivation

Several studies were considered as a potential basis for the RfD. These select studies are summarized briefly in **Table 8-2**.

Table 8-2. Studies Considered for Ethylbenzene RfD Derivation

Endpoints	Species	Reference	NOAEL	LOAEL
Liver and blood effects	Rat	Mellert <i>et al.</i> (2004, 2007)	75 mg/kg bwt/day	250 mg/kg bwy/day
Ototoxicity (inhalation)	Rat	Gagnaire <i>et al.</i> (2007)	200 ppm (audiometric threshold) Not determined (outer hair cell loss)	400 ppm (audiometric threshold) 200 ppm (outer hair cell loss, LOEL)
Liver, pituitary (inhalation)	Mouse	NTP (1999)	75 ppm	250 ppm

8.2.3.1 Mellert *et al.* (2004, 2007)

Key Findings

Male and female Wistar rats were subchronically exposed for three months to 75, 250, or 750 mg ethylbenzene/kg bwt/day, administered in corn oil in two equal doses given 8 hours apart (Mellert *et al.*, 2004, 2007). The key effects in male rats were **liver effects** (hypertrophy). Key effects in the female rat were **liver effects** (hypertrophy), **mild regenerative anemia** (increase in mean corpuscular volume [MCV]), and decreased **prothrombin time**, with a **NOAEL 75 mg/kg**. It should be noted that effects on MCV and clotting were not identified in subchronic and chronic inhalation studies in rats (albeit, in a different strain) (NTP, 1999).

Proposed MOA and Internal Dose Metrics

Proposed MOA and Internal Dose Metrics for Liver Effects

The MOA for **liver effects** in male rats is likely similar to the mouse liver cancer and noncancer MOA (discussed above). Again, if related to phenobarbital-like MOA, the relevance to human health remains uncertain.

Proposed MOA and Internal Dose Metrics for Regenerative Anemia and Prothrombin Time

The MOA(s) for **regenerative anemia and prothrombin time** have not been determined. In the absence of other information, potential internal dose metrics generally applicable to hazard assessment include (1) AUC for parent compound in a relevant tissue or blood and (2) amount metabolized (Kirman *et al.*, 2003). The amount metabolized can be normalized to either the tissue in which it is generated, if that is the target tissue, or the whole body. Since the target tissue(s), bone marrow and/or spleen are richly perfused tissues lacking any known or anticipated capability for ethylbenzene metabolism, the most relevant dose metrics are AUC in richly perfused tissues and amount metabolized normalized to bodyweight.

Proposed Uncertainty Factors

- A **UFL** of 1 is appropriate because the key study identified a NOAEL for increases in regenerative anemia and prothrombin time.
- A **UFS** of 10, the default value, is proposed because the key oral study is a subchronic study, rather than a chronic study.
- A **UFA** of 3 is proposed. As noted above (Section 8.2.2.4), the default UFA of 10 can be divided into pharmacokinetic and pharmacodynamic components each equal to a factor of ~3. Validated PBPK models appropriate for the interspecies extrapolation of total metabolism, liver metabolism, or liver concentration of ethylbenzene in rats and humans are available (see Appendix P), so the pharmacokinetic component of UFA can be set equal to 1. We recommend retention of the full factor of 3 for the pharmacodynamic portion of UFA.
- A **UFH** of 10, the default value is proposed. Because low-dose metabolism of orally administered ethylbenzene is essentially complete (little is exhaled; the rest is metabolized), sensitivity analyses for humans indicate that this value of UFH is likely to provide adequate for protection of children (Appendix P).
- An uncertainty factor for database sufficiency (**UFD**) = 1 is proposed because ethylbenzene has been extensively tested by the inhalation route, as noted above. The findings by the inhalation route were also used to derive potential RfD values (Section 8.2.2.3), using a PBPK model for route-to-route and interspecies extrapolation, increasing confidence that the potential RfDs are derived from an adequate testing database.

Therefore, a composite UF of 300 (1x10x3x10x1) is recommended for the ethylbenzene RfD.

8.2.3.2 RfD Derivations from an Oral Study

RfD Derivation for Liver Effects Observed in an Oral Study

BMDS Analysis of Liver Effects

Based on the similarity of the responses in males and females, the data sets were combined. As discussed above, because it is expected that PBPK modeling should resolve nonlinearity when an appropriate internal dose measure is used, this observation offers empirical support against the use of parent chemical in the target tissue in the dose response assessment. The fit for AM/VL as the dose metric was consistently better than when AUCL was used as the dose metric (see Appendix Q for details). The best fit for AM/VL was generated using the quantal-quadratic model (AIC = 61.4, p = 0.576), resulting in an LED10 of 1,546 mg ethylbenzene metabolized/kg liver/day.

RfD Derivation for Liver Effects

Dividing the LED10 (1,546 mg ethylbenzene metabolized/kg liver/day) by the composite UF of 300 yielded a target human internal dose (AM/VL) of 5.16 mg ethylbenzene metabolized/kg liver/day. Daily AM/VL was calculated as the difference between the AM/VL for 360 hours (15 days) and AM/VL for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfD for liver effects** was 0.15 mg/kg bwt/day, which rounds to **0.2 mg/kg bwt/day**.

RfD Derivation for Regenerative Anemia

BMDS Analysis of Mean Corpuscular Volume (MCV)

No acceptable fits were derived when AUCR was used as the dose metric. An acceptable fit was derived for AM/BW as the dose metric using the Linear dose-response model (AIC = 50.9, p = 0.212, see Appendix Q for details). An LED10 of 122.1 mg ethylbenzene metabolized/kg bwt/day was derived from the linear model.

RfD Determination for Mean Corpuscular Volume

Dividing the LED10 (122.1 mg ethylbenzene metabolized/kg bwt/day) by the composite UF of 300 yielded a target human internal dose (AM/BW) of 0.407 mg ethylbenzene metabolized/kg bwt/day. Daily AM/BW was calculated as the difference between the AM/VL for 360 hours (15 days) and AM/VL for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfD for MCV** was 0.44 mg/kg bwt/day, which rounds to **0.4 mg/kg bwt/day**.

RfD Derivation for Decreased Prothrombin Time

BMDS Analysis of Decreased Prothrombin Time

Superior fits were derived when AM/BW was used as the dose metric rather than AUCR. The best fit was derived for AM/BW as the dose metric using the Linear dose-response model (AIC = 76.8, $p = 0.712$, see Appendix Q for details). An LED10 of 157.1 mg ethylbenzene metabolized/kg bwt/day was derived from the Linear model.

RfD Determination for Decreased Prothrombin Time

Dividing the LED10 (157.1 mg ethylbenzene metabolized/kg bwt/day) by the composite UF of 300 yielded a target human internal dose (AM/BW) of 0.524 mg ethylbenzene metabolized/kg bwt/day. Daily AM/BW was calculated as the difference between the AM/VL for 360 hours (15 days) and AM/VL for 336 hours (2 weeks) to ensure establishment of steady state in the PBPK model. The PBPK-model derived **RfD for prothrombin time** was 0.57 mg/kg bwt/day, which rounds to **0.6 mg/kg bwt/day**.

8.2.3.3 RfD Derivation from Inhalation Studies

Since the testing database for ethylbenzene is more extensive for inhalation studies, consideration should be given to deriving an RfD based on inhalation studies. The target internal dose metrics for ototoxicity and liver effects that were used to derive RfCs were also used to derive potential RfDs using the human PBPK model and assuming continuous ingestion. The resulting potential RfDs were as follows:

- Ototoxicity RfD: 0.5 mg/kg bwt/day
- Liver effects RfD: 0.5 mg/kg bwt/day (from chronic mouse study)
- Pituitary hyperplasia RfD: 2.9 mg/kg bwt/day.

Considering the different studies and routes of exposure used to support these analyses, the lowest potential RfD values are remarkably consistent.

8.2.3.4 Proposed RfD

The hepatic effects seen in the chronic mouse inhalation study (NTP, 1999) and subchronic oral rat study (Mellert *et al.*, 2004, 2007) were similar. Use of the mouse inhalation study rather than the rat oral study obviates the need for an uncertainty factor for study duration (subchronic to chronic extrapolation) and increases confidence because the inhalation toxicity testing database is more extensive than the oral database. Other effects observed in the oral subchronic study are of questionable significance because they have not been seen in chronic studies of the same species. Also, the potential RfDs based on these effects are similar to the RfD derived for liver toxicity by inhalation. The **RfD of 0.5 mg/kg bwt/day**, based on liver effects observed in the chronic mouse inhalation study, is proposed as the noncancer reference value for oral exposure to ethylbenzene.

8.2.3.5 Discussion of Proposed RfD

If benchmark dose analysis and PBPK modeling were not used, the default RfD would likely be derived as follows:

Oral NOAEL = 75 mg/kg bwt/day

Duration adjustment: none

Uncertainty factors: same as above, except UFA = 10, resulting in a composite UF of 1000

$$\text{Default RfD} = 75 \times (36/168)/1000 = 0.08 \text{ mg/kg bwt/day}$$

The existing RfD of 0.1 mg/kg bwt/day (IRIS, 1988) was based on liver and kidney toxicity observed in a 182-day (subchronic) study in which a NOAEL of 136 mg/kg/day (5 days/week) was identified (LOAEL = 408 mg/kg bwt/day, 5 days/week) (Wolf *et al.*, 1956). We interpreted this study to be unreliable, as discussed in the Robust Summaries (Appendix O). A total uncertainty factor of 1000 (UFA = 10, UFH = 10, UFS = 10) was applied, and a dosing schedule adjustment was made to arrive at the existing RfD. U.S. EPA assigned low confidence to the study, the database, and the RfD.

Evaluation of a more recent oral, subchronic study in rats and inhalation studies yielded potential RfDs ranging from 0.2 to 2.9 mg/kg bwt/day, and a proposed RfD of 0.5 mg/kg bwt/day. The reasons for the difference, as compared to the existing RfD, were the use of a different, chronic study, use of benchmark dose modeling (rather than the NOAEL) to identify the point of departure, use of a PBPK model for route-to-route extrapolation, and use of a PBPK model for interspecies extrapolation rather than the default UFA.

While the RfD for another endpoint, ototoxicity, is the same as the proposed liver RfC, we consider the uncertainty factors and response level selected for ototoxicity (in particular, the UFS of 3) to be highly conservative. The concordance between the effects by inhalation vs. oral is also greater for the liver toxicity endpoint, verifying that it should be the endpoint of concern for oral exposures.

Overall, the confidence in the proposed RfD is medium-to-high. The toxicity-testing database is extensive, indicating that it is unlikely that there are toxicologically important effects of ethylbenzene that have not been identified. The confidence in the PBPK-derived internal dosimetry estimates for the range used in liver dose response is high. Confidence in the key study is considered high because it is well designed, and assessed a number of toxicological endpoints following lifetime exposures to ethylbenzene. Confidence in the dose-response modeling is considered to be medium since several dose-response models were found to provide an acceptable fit to the data.

8.2.4 Conclusion

The existing RfC and RfD for ethylbenzene were determined in 1991 and 1988 and are both based on relatively old studies (1981 and 1956, respectively) that have been

superseded by newer developmental toxicity and oral subchronic studies. In addition, the overall database for testing of ethylbenzene is more extensive than it was at the time U.S. EPA determined the existing RfC and RfD, with the additional conduct of a multigeneration reproduction study, immunotoxicity, adult and developmental neurotoxicity, ototoxicity, and chronic toxicity studies, as well as pharmacokinetic studies and development of PBPK models. The interpretation of the studies has also been enhanced by the development of Benchmark Dose/Benchmark Concentration analysis. The additional information and techniques currently available for RfC and RfD determination allowed the development of new proposed toxicity reference values with greater confidence than could be associated with the existing values. The proposed **RfC of 0.3 ppm** and **RfD of 0.5 mg/kg/day** are recommended as the basis for risk assessment of human exposures to ethylbenzene.

8.3 Cancer Dose-Response Assessment for Ethylbenzene

Currently, ethylbenzene is considered not classifiable as to human carcinogenicity (Group D) by U.S. EPA (IRIS, 1991). However, since the time of U.S. EPA's assessment, cancer bioassays have been conducted for ethylbenzene in both rats and mice (NTP, 1999). In these studies, the incidence of several tumor types was increased in rodents following lifetime exposures to ethylbenzene (NTP, 1999), suggesting that the carcinogenic potential of ethylbenzene needs to be reevaluated.

A dose-response assessment was conducted for ethylbenzene with the purpose of deriving a cancer potency estimate. This assessment was conducted with consideration of U.S. EPA's framework described in its *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005e).

8.3.1 Methods

The published literature was reviewed regarding the toxicity, carcinogenicity, toxicokinetics, and mode of action for ethylbenzene. This information was used to prepare the following sections:

- **Section 8.3.2:** This section provides a summary of the cancer hazard identification presented previously in **Section 7.9**.
- **Section 8.3.3:** This section provides an assessment of the modes of action (MOAs) by which ethylbenzene produces tumors in laboratory rodents. This information is used to ascertain the relevance of these tumors to human health, as well as to help guide key decisions made in the dose-response assessment. Frameworks for evaluating the cancer mode of action (Meek *et al.*, 2003; USEPA, 2005; Boobis *et al.*, 2006) were considered in this analysis.
- **Section 8.3.4:** This section contains the dose-response assessment conducted for ethylbenzene. This assessment includes the use of internal dose measures as determined by a PBPK model. PBPK models have been developed to describe the pharmacokinetics of ethylbenzene in mice, rats, and humans (for details, see

Appendix P). The following decision points were considered in the dose-response assessment.

1. *Data Set* – Studies from the published literature and unpublished laboratory reports were reviewed to identify possible data sets to serve as the basis for ethylbenzene cancer potency, as well as supporting information regarding kinetics and mode of action.
2. *Dose Measure* – The dose-response assessment was conducted using a PBPK-derived internal dose measure. An appropriate internal dose measure was selected based upon a consideration of the MOA. All PBPK modeling was performed using Advanced Continuous Simulation Language (ACSL, version 11.8 from Aegis Technology Group).
3. *Dose-Response Model* – The dose-response relationship for ethylbenzene - induced tumors was assessed in terms of extra risk. All dose-response modeling was performed using U.S. EPA's Benchmark Dose Software (BMDS, version 1.3.2).
4. *Point of Departure* – An appropriate point of departure was selected based upon a consideration of the range of observations defined by the critical data set.
5. *Low-Dose Extrapolation* – Information regarding the MOA for ethylbenzene - induced tumors was used to identify potential sources of nonlinearity in the dose-response relationship.
6. *Cancer Value Presentation* – Consistent with U.S. EPA guidelines (U.S. EPA, 2005e), cancer potency estimates included characterization of central tendency, upper bound, and lower bound values.

8.3.2 Cancer Hazard Identification Summary

Information regarding the potential carcinogenicity of ethylbenzene from epidemiology studies is limited and therefore uninformative for human risk. In a study of approximately 200 (exact number not specified) ethylbenzene production workers in Czechoslovakia (mean length of employment of 12.2 years between 1964 and 1985), no tumors were reported over the previous 10-year period (Bardodej and Cirek, 1988). However, the results of this study are limited by insufficient details presented, small number of workers, and relatively short follow-up time period. In a study of 560 styrene production and polymerization workers exposed to ethylbenzene and other chemicals (styrene, benzene, toluene), 17 cancer deaths were reported vs. 21 cancer deaths expected (Nicholson *et al.*, 1978).

Information collected in laboratory animals indicates that lifetime exposures to high concentrations of ethylbenzene can produce tumors in multiple tissue sites. In the key study, groups of 50 male and 50 female F344/N and B6C3F1 mice were exposed to either 0, 75, 250, or 750 ppm ethylbenzene via inhalation for 6 hours/day, 5 days/week for 104 weeks (NTP, 1999). In male rats, survival was significantly reduced in animals exposed to the highest concentration. Significant increases were observed for the incidence of several tumor types at the highest test concentration: (1) renal tubule adenoma and carcinoma (combined) in male rats, and renal tubule adenomas in female rats; (2) alveolar/bronchiolar adenoma and carcinoma (combined) in male mice; (3) hepatocellular

adenoma and carcinoma (combined) in female mice; and (4) Leydig cell tumors (LCT) in male rats; (**Table 8-3**). Significant nonneoplastic effects were also observed in target tissues including chronic progressive nephropathy (CPN) and renal tubule hyperplasia in the kidneys, hyperplasia/metaplasia in the lungs, and eosinophilic foci in the liver.

A cancer bioassay was conducted for 1-phenylethanol, a principal metabolite of ethylbenzene (NTP, 1990). In this study, groups of 50 male and 50 female F344/N and B6C3F1 mice were exposed to 0, 375, or 750 mg/kg-day 1-phenylethanol via corn oil gavage for 5 days/week for 103 weeks. No increase in tumor incidence was observed in mice of either sex, or in female rats. Renal tubule adenomas were significantly increased in male rats exposed to the highest dose.

Groups of 50 male and 50 female Sprague-Dawley rats were exposed to 0 or 800 mg/kg-day ethylbenzene via oil gavage for 4 days/week up to 104 weeks, with remaining animals sacrificed at week 123 (Maltoni *et al.*, 1985, 1997). In a second experiment, groups of 40-50 male and 40-50 female Sprague-Dawley rats were exposed to 0 or 500 mg/kg-day ethylbenzene via oil gavage for 4 days/week up to 145 weeks. Survival was affected in all treated animals. Following exposure to 800 mg/kg-day, a small increase in the incidence of nasal and oral tumors was reported in female rats, and a borderline increase in neuroesthesioepitheliomas was reported in male rats.

8.3.3 Mode of Action

An evaluation of the mode of action (MOA) by which ethylbenzene produces tumors in rodents was conducted with consideration of frameworks created by USEPA and ILSI RSI/IPCS (Meek *et al.*, 2003; USEPA, 2005; Boobis *et al.*, 2006). In these frameworks, three fundamental questions are considered for the MOA:

1. *Is the weight of evidence sufficient to establish an MOA in animals?*
2. *Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?*
3. *Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?*

Following a consideration of these three questions, a confidence statement is given, along with a discussion of the implications of the MOA to the risk assessment.

The MOAs by which each of the rodent tumor types identified in **Section 8.3.2** (rat kidney, mouse liver, mouse lung, and rat testes) are summarized in **Table 8-4** and are discussed below. This discussion includes a consideration of a default MOA, direct genotoxicity, found not to be applicable for all tumor types and associated proposed MOAs (**Section 8.3.3.5**). Because the proposed MOAs for ethylbenzene involve the formation of metabolites, the metabolic pathways for side-chain oxidation (**Figure 8-1**, Box A) and ring oxidation (**Figure 8-1**, Box B) are provided (Bus, 2006).

Table 8-3. Summary of Key Neoplastic and Nonneoplastic Effects of Ethylbenzene in Rats and Mice (NTP, 1999; Chan *et al.*, 1998)

Species	Sex	Concentration (ppm)	Kidney	Testes		Lung		Liver		
			Chronic Progressive Nephropathy (mean severity score)	Adenoma/ carcinoma*	Hyperplasia	Adenoma	Hyperplasia/ Metaplasia	Alveolar/ broncholar adenoma/ carcinoma	Eosinophilic Foci	Adenoma/ carcinoma
Rat	M	0	47/50 (2.3)	3/50	14/50	36/50	2/50	3/50	5/50	0/50
		75	43/50 (2.4)	5/50	19/50	33/50	2/50	1/50	11/50	3/50
		250	47/50 (2.3)	8/50	12/50	40/50	1/50	0/50	4/50	0/50
		750	48/50 (3.5)	21/50	8/50	44/50	2/50	1/50	9/50	0/50
	F	0	38/50 (1.3)	0/50	NA	1/50	1/50	2/50	0/50	
		75	42/50 (1.6)	0/50		5/50	1/50	3/50	0/50	
		250	43/50 (1.7)	1/50		2/50	1/50	8/50	0/50	
		750	46/49 (2.3)	8/49		5/49	0/49	5/49	0/49	
Mouse	M	0	34/50	0/50	0/50	1/49	1/50	7/50	6/50	29/50
		75	38/50	0/50	1/50	0/50	6/50	10/50	8/50	25/50
		250	40/50	1/50	0/50	1/50	4/50	15/50	8/50	30/50
		750	36/50	0/50	0/50	1/50	10/50	19/50	12/50	28/50
	F	0	13/50	0/50	NA	0/50	4/50	5/50	13/50	
		75	7/50	0/50		1/50	6/50	7/50	12/50	
		250	9/50	0/50		3/50	5/49	6/50	15/50	
		750	21/50	0/50		1/50	8/50	22/50	25/50	

*Standard and extended evaluations combined; bolded tumor incidence values indicated a statistically significant increase over untreated control animals.

NA – not applicable

Table 8-4. Comparison of Possible Modes of Action for Ethylbenzene-Induced Tumors

Group	Criteria	Proposed MOAs for Specific Tumors				Default MOA for All Tumors
		Rat Kidney (Nongenotoxic)	Mouse Liver (Nongenotoxic)	Mouse Lung (Nongenotoxic)	Rat Testes (Nongenotoxic)	(Direct Genotoxicity)
Hill Criteria	Strength of Association	+	+	+	+/-	<i>in vivo</i> - <i>in vitro</i> +/-
	Consistency of Association	+	+	+	-	-
	Specificity of Association	+	+	+	+/-	-
	Dose-Response Concordance	+	+	+	-	+/-
	Temporal Relationship	+	+	+	+/-	+/-
	Coherence and Plausibility	+	+	+	+/-	-
Relevance to Humans	Qualitative Differences	Not Relevant	Not Relevant	Relevance assumed	Not Relevant	NA
	Quantitative Differences	NA	NA	PK differences observed; PD differences likely	PK differences observed; PD differences likely	NA

“+” = Data available to support mode of action; “-“ = Data available to refute mode of action; “+/-“ = Data are equivocal; NA = Not applicable

8.3.3.1 Proposed MOA for Kidney Tumors

Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

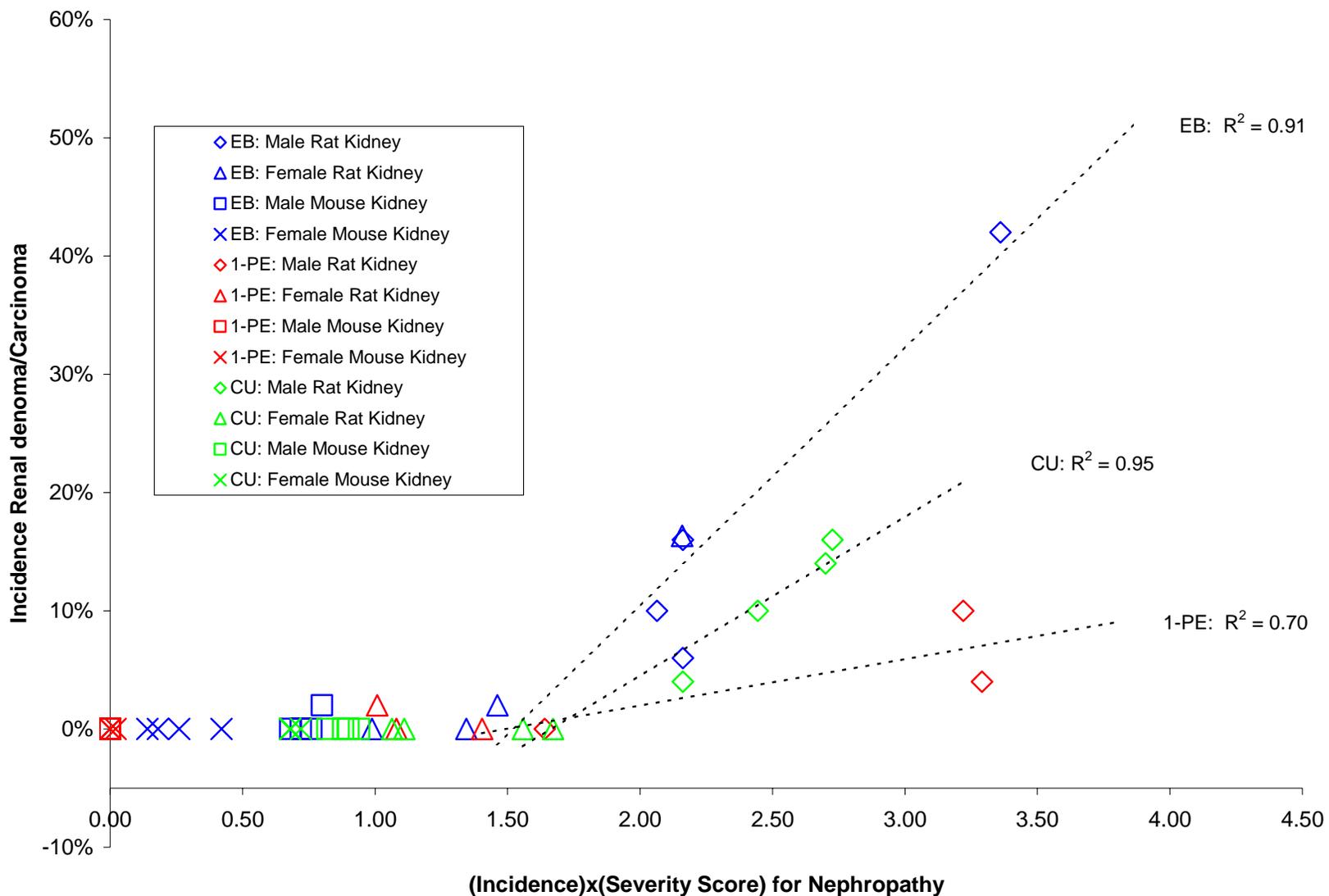
After exposure and systemic absorption of ethylbenzene, the MOA for kidney tumors in rats is proposed to include the following key events: (1) absorption and distribution of ethylbenzene to tissue; (2) metabolism of ethylbenzene in liver and lung to 1-phenylethanol; (2) distribution of 1-phenylethanol to kidney; (3) interaction between 1-phenylethanol and critical cellular targets (*e.g.*, α_{2u} -globulin); (4) exacerbation of chronic progressive nephropathy (CPN); and (4) progression to cancer. The weight of evidence for this MOA is considered below within the context of the modified Hill criteria below.

- *Strength of Association* – Following chronic inhalation exposure to ethylbenzene, the development of renal tumors along with an increased incidence and severity of CPN has been observed in male rats (Hard, 2002). Following ethylbenzene exposure, advanced severity of CPN, the location of the lesions in kidneys with the highest severity of CPN, the increased proportion of atypical tubule hyperplasias and adenomas, and the increase in proliferative lesions are all features associated with CPN induced kidney tumors (Hard, 2002). Therefore, it was concluded that kidney tumors were induced through exacerbation of rat CPN (Hard, 2002). According to a review by Wolf and Mann (2005), the data described by Hard (2002) suggest a direct correlation between ethylbenzene enhanced CPN and the development of renal tumors. It has been shown that following ethylbenzene exposure, there is an accumulation of α_{2u} -globulin early after exposure, but deposition of α_{2u} -globulin soon decreases; however, S-phase DNA synthesis and histopathological changes continue to increase (Stott *et al.*, 2003). This indicates that renal tumors may arise from CPN and a more chronic cell proliferation. The possible involvement of α_{2u} -globulin in accentuating CPN is suggested, however, in that additional subchronic studies provide evidence of hyaline droplets, a sign of α_{2u} -globulin accumulation, in proximal tubules (Stott *et al.*, 2003; Mellert *et al.*, 2004). α_{2u} -Globulin has previously been associated with renal tumors in the male rat. However, one of the histological criteria of α_{2u} -globulin tumors, granular casts and papillary mineralization, were absent in the ethylbenzene chronic bioassays (Hard, 2002). The kidney tumor response was significantly less in female rats, and was only observed when special step-sectioning histological examinations were performed. It is uncertain if all chemicals that produce tumors via an α_{2u} -globulin-type mode of action might cause a similar weak tumor response in female rats that would only be revealed by step-sectioning. 1-Phenylethanol is one of the primary metabolites of ethylbenzene in rats (Engstrom, 1984) and has been shown to be weakly carcinogenic with regards to the kidney and has increased the occurrence of CPN (NTP, 1990). Based upon the rodent data for incidence and severity in **Table 8-3**, a strong correlation can be observed between the CPN and renal tumors, which appears to hold across species and sex (**Figure 8-2**). Regardless of sex or species, this plot suggests that kidney tumors are only observed when the product of incidence and severity score for CPN exceeds a value of approximately 1 (*e.g.*,

100% x 1), with no evidence of a correlation below this value. The strong correlation between CPN and kidney tumors, when the product of incidence and severity score for CPN exceeds 1, provides support for this MOA.

- *Consistency of Association* – A consistent association between CPN and kidney tumors is apparent for ethylbenzene across species and sex (**Figure 8-2**). Studies have provided evidence that ethylbenzene exposure results in kidney tumors in rats along with an exacerbation of rat CPN (Hard, 2002; Stott *et al.*, 2003; NTP, 1999). Also, increases in hyaline droplets have been noted within tubules of male rats following ethylbenzene exposure (Stott *et al.*, 2003; Mellert *et al.*, 2004), suggesting the possibility of a weak α_{2u} -globulin accentuation of the CPN mode of action. Therefore, this MOA demonstrates consistency in the association between CPN and kidney tumors.
- *Specificity of Association* – Renal tumors have been reported in male and female rats after exposure to ethylbenzene, but not in male and female mice (NTP, 1999; Chan *et al.*, 1998). It has been reported that the kidney weights of male rats following ethylbenzene exposure have increased (Stott *et al.*, 2003) which is indicative of treatment-related toxicity. To a lesser extent, female rats had a slight elevation in occurrence of renal tumors (0% vs. 0%, 2%, and 16% for controls and exposed animals, respectively) (NTP, 1999; Chan *et al.*, 1998). However, this increase in female renal tumors was only observed following a histopathology step section (extended) evaluation. Under standard histopathological methods, the occurrence of renal tumors in female rats was only 0% vs. 0%, 0%, and 2% for controls and exposed animals, respectively (NTP, 1999; Chan *et al.*, 1998). Likewise, the occurrence of end-stage CPN in male rats was 42% of the high dose group and only 12% of the control group (Hard, 2002; Hard and Khan, 2004). In contrast, female rats showed an occurrence of end-stage CPN in only 8% of the high dose animals versus 0% in control animals (Hard, 2002; Hard and Khan, 2004). Additionally, in a review by Abrass (2000), a study performed by Baylis (1994) reported that castrated male rats were protected from CPN; however, castrated female rats showed the same rate of CPN. It was concluded that the data from Baylis (1994) implied that male sex steroids contribute to CPN (Abrass, 2000), which is consistent with observations that α_{2u} -globulin is regulated by male sex steroids. Because the incidence of CPN was generally greater than 80% in male and female rats, and was generally less than 80% in male and female mice (**Figure 8-2**), this MOA serves to explain the species- and sex-specific responses observed for kidney tumors in rodents exposed to ethylbenzene.

Figure 8-2. Correlation Plot for Kidney Lesions in Rats and Mice* Exposed to Ethylbenzene



- Dose-Response Concordance* – Following chronic inhalation exposure of 750 ppm of ethylbenzene, an increased incidence of kidney tumors in rats was observed (42% vs. 6% controls) (Chan *et al.*, 1998; NTP, 1999). The severity scores for CPN also demonstrated a dose-dependent trend, with average severity scores increasing from 5.7 to 7.4 in male rats (on a scale from 0 to 8 rather than the 0 to 4 scale used by NTP in **Table 8-3**), and from 3.8 to 5.5 in female rats (Hard, 2002). Chronic exposures to 750 ppm of ethylbenzene increased the occurrence of end-stage CPN in rats (68% at the high dose group vs. 12% in the control group) (Hard, 2002; Hard and Khan, 2004). After subchronic oral exposure of 250 and 750 mg/kg, an increase in absolute and relative kidney weights in rats was reported (Mellert *et al.*, 2004, 2007). 1-Phenylethanol, a metabolite of ethylbenzene, has increased the incidence of renal tumors at 375 and 750 mg/kg by gavage (13/41 (32%) and 14/28 (50%), respectively) and increased the incidence of severe end-stage CPN at 375 and 750 mg/kg (33/50 (66%) and 33/50 (66%), respectively) (NTP, 1990). Inspection of the dose-response data in **Table 8-3** indicate that when inhalation exposures to ethylbenzene were sufficient to increase the incidence of CPN above 80%, a corresponding increase in kidney tumors was also observed. Therefore, this MOA offers concordance with the dose-response relationship observed for kidney tumors.
- Temporal Relationship* – After two years exposure to ethylbenzene, kidney tumor incidences were increased (Chan *et al.*, 1998; NTP, 1999). Effects on the kidney, however, are observed at much earlier time points. Following 13 weeks of ethylbenzene exposure (750 and 1000 ppm), male rats developed mild and/or low-moderate CPN, whereas controls only developed minimal CPN (Hard, 2002). However, CPN becomes more severe following 2-year exposures of rats to ethylbenzene (NTP, 1990). An increase in α_{2u} -globulin accumulation and hyaline droplets has been observed as early as one or four weeks following inhalation exposure of ethylbenzene (Stott *et al.*, 2003). Subchronic exposure by gavage has shown an increase in kidney weight and hyaline droplets in male rats (Mellert *et al.*, 2004, 2007). Also, following a 6-hour exposure of ethylbenzene (up to 600 ppm) the major metabolite was identified as 1-phenylethanol (Engstrom, 1984), which has also been shown to accentuate rat CPN (NTP, 1990). Therefore, this MOA is consistent with the temporal relationship observed for kidney tumors.
- Biological Plausibility and Coherence* – Accentuation of CPN is a biologically plausible MOA for kidney tumors following exposure to ethylbenzene. However, CPN is a rodent specific disease and considered irrelevant for extrapolating this MOA to humans (Hard, 2002; Hard and Khan, 2004). Additionally, structurally similar chemicals, 1-phenylethanol and hydroquinone, have shown to cause an exacerbation of CPN in male rats (NTP, 1990; Hard and Khan, 2004). Therefore, this MOA is biologically plausible and coherent.

Are Key Events in the Animal MOA Plausible in Humans?

A role for CPN in kidney tumors is supported by observations made for structurally similar chemicals (1-phenylethanol, cumene), in which an increased incidence of kidney tumors are observed in male rats (for which the incidence of CPN exceeds 80%), but not in female rats or in mice of either sex (NTP, 1990, 2007).

CPN can be exacerbated by chemical exposure, leading to an increase in incidence and average severity of the spontaneous disease. In addition, slight or marginal increases in renal tubule hyperplasia and/or adenoma often accompany an increase in chemically exacerbated CPN. Substantiating the link between an increase in renal tubule tumors in a carcinogenicity bioassay, and CPN as the underlying prime influence, requires fulfillment of a set of specific criteria (Lock and Hard, 2002):

1. The chemical must cause an exacerbation of CPN to the most advanced grades, mostly to end-stage CPN, which is a terminal condition resulting in renal failure because almost no normal parenchyma remains. Renal histopathology in the NTP bioassay for ethylbenzene was recently reevaluated (Hard, 2002), with the finding that ethylbenzene caused a severe exacerbation of CPN in the high-dose male rats, such that 68% had an end-stage grade of severity compared to only 12% in the control group.
2. The tumors must be of marginal or low incidence, predominantly adenomas of small size, or incipient neoplasms borderline with atypical tubule hyperplasia, occurring in rats with an end-stage, or at least very severe, grade of CPN. Kidney tumors in male rats exposed to ethylbenzene were predominantly adenomas, while those in female rats were exclusively adenomas (NTP, 1999)
3. The proliferative lesions must arise within CPN-affected tissue, and are not infrequently bilateral or multiple, particularly the foci of atypical tubule hyperplasia. In addition, the small lesions are not restricted in their distribution to either cortex or the outer strip of the outer medulla, contrary to the situation when tumors are associated with a specific site of renal tubule injury. Reevaluation of the histopathology for ethylbenzene revealed that all of the tumors were located within CPN-affected parenchyma (Hard, 2002).

Although rodents and humans share some key events in the MOA including formation of 1-phenylethanol metabolite (see **Table 8-5**), critical qualitative differences between rats and humans exist. Of the major human renal diseases (renal vascular disease due to hypertension; diabetes; glomerulonephritis; infective obstructive nephropathy), none have the singular features of CPN in the laboratory rat (Hard and Khan, 2004). In contrast to humans, rats are characterized by the early appearance of proteinuria and maintenance of a normal glomerular filtration rate until very advanced age (Rodriguez-Puyol, 1998). In the laboratory rat, CPN progresses relentlessly as the animal ages, occurring with virtually a 100% incidence by two years of age. Although there is an increase in sclerotic glomeruli in humans, involving as many as 30% of nephrons in the eighth decade with presumed loss of filtering surface (Kaplan *et al.*, 1975), no specific kidney disease that is totally confined to the aging kidney has been identified in humans (Frocht and Fillit,

1984). Whereas progression of CPN in the rat can be ameliorated by a reduction in dietary protein, the prevailing view is that diseases causing chronic renal failure in man show negligible benefit from a low-protein diet (Ruggenti *et al.*, 2001). In terms of histopathology, none of the human renal diseases are characterized by the same spectrum of change as CPN. In contrast to some of the human renal diseases, CPN is not an inflammatory or vascular disease, nor does it have an immunological or autoimmune basis, and hematuria is not a clinical finding (Hard and Khan, 2004). Relative to the various human causes of end-stage renal disease, the various features of CPN indicate that it has no strict counterpart in humans. Additionally, there is a potential role for α_2 -globulin accumulation in the male rat kidney (Stott *et al.*, 2003), which may explain the relative sensitivity between male and female rats to ethylbenzene, but more importantly is a process that does not occur in humans. Together, these data suggest that the rat kidney tumors associated with chronic ethylbenzene exposure are not qualitatively relevant to human health, and should not be used as a basis for quantitative risk assessment.

Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans?

This question is moot because the weight of evidence for the postulated mode of action for carcinogenesis in animals is not relevant in humans due to qualitative species differences. However, quantitative differences do exist between rats and humans and include: (1) rodent exposures are orders of magnitude higher than expected human exposure; (2) kidney tumor responses were limited to the high exposure only (750 ppm), a concentration well above non-linear pharmacokinetic behavior; (3) the rates of metabolism of ethylbenzene in liver and lung microsomes are higher in rats than in humans (Saghir *et al.*, 2006); and (4) the background incidence of kidney tumors is greater in male rats than in female rats, which in turn is greater than the background incidence in humans.

Table 8-5. Key Events in the MOA for Ethylbenzene-Induced Kidney Tumors

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Exposure	Exposures to EB occur under controlled conditions in a laboratory setting (NTP, 1999)	Exposures to EB can occur at the workplace, from consumer products, and from the environment	Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals	Rodent exposures are orders of magnitude higher than expected human exposure	None identified. Linearity is assumed.
Absorption	EB is well absorbed following inhalation (Chin <i>et al.</i> , 1980) or ingestion (El Mastri <i>et al.</i> , 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsurata, 1982; Morgan <i>et al.</i> , 1991; Susten <i>et al.</i> , 1990).	EB is well absorbed following inhalation (Bardodej and Bardodejova, 1970; Engström and Bjurström, 1978; Åstrand <i>et al.</i> , 1978; Gromiec and Piotrowski 1984). and dermal (Dutkiewicz and Tyras, 1967) routes. EB is assumed to be well absorbed via ingestion.	None identified. The process dictating absorption are assumed to qualitatively similar.	None identified. The process dictating absorption are assumed to quantitatively similar.	None identified. Linearity is assumed.
Distribution of parent chemical to the liver	Absorbed EB is rapidly distributed to all tissues in the body (Chin <i>et al.</i> , 1980; Engström <i>et al.</i> , 1985; Cappaert, 2000)	Rapid distribution of EB to human tissues is assumed.	None identified. The process dictating absorption are assumed to qualitatively similar.	None identified. The process dictating absorption are assumed to quantitatively similar.	

Metabolism to active metabolite	Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of EB (McMahon and Sullivan, 1966, 1968; Engström, 1984; Kaubisch <i>et al.</i> , 1972 Stott <i>et al.</i> , 2003; Saghir <i>et al.</i> , 2006)	Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of EB (Engström <i>et al.</i> , 1984; Sams <i>et al.</i> , 2004; Saghir <i>et al.</i> , 2006)	None identified. The processes dictating metabolism are assumed to be qualitatively similar.	Rates of metabolism of EB in lung microsomes exhibit clear species differences: mice > rats ≥ humans (Saghir <i>et al.</i> , 2006)	Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation)
Distribution of active metabolite to kidney	Distribution of metabolites to all tissues including the kidneys is assumed	Distribution of metabolites to all tissues including the kidneys is assumed	None identified. The process dictating distribution are assumed to qualitatively similar.	None identified	None identified. Linearity is assumed
Detoxification/Elimination of active metabolite	In rats, about 83% of EB was excreted in the urine over 72 hours; ~8% was exhaled; ~0.7% was excreted in the feces; ~0.03% in exhaled CO ₂ ; ~8.2% in expired gases; ~0.2% remained in the tissues (Chin <i>et al.</i> , 1980).	Absorbed EB is excreted in the urine and exhaled breath (Bardodej and Bardodejova, 1970; Åstrand <i>et al.</i> , 1978; Engström <i>et al.</i> , 1984; Gromiec and Piotrowski, 1984; Kawai <i>et al.</i> , 1992; Knecht <i>et al.</i> , 2000)	None identified. The process dictating elimination are assumed to qualitatively similar.	Species differences in urinary metabolites reflect differences in metabolism	Altered elimination is possible due to kidney damage
Exacerbation of CPN	EB produces a dose-dependent increase in the incidence and severity of CPN in male and female rats (NTP, 1999)	No evidence	Rodent CPN has no counterpart in humans (Hard, 2002). Potential involvement of α _{2u} -globulin in male rats, a process which does not occur in humans.	None identified	Threshold for ethylbenzene-induced CPN of sufficient incidence (~80%) and severity to result in tumors
Promotion of kidney tumors				Background incidence of kidney tumors generally male rats > female rats > male mice > female mice > humans	

Conclusion

Confidence in the proposed MOA is high since it is well supported by available studies. Following exposure to ethylbenzene, evidence exists to support the MOA of kidney tumors in rats resulting from an increased incidence of CPN by a primary ethylbenzene metabolite, 1-phenylethanol. The MOA may also include a possible weak accentuation of CPN by involvement of α_{2u} -globulin in male rats. Key events following exposure to ethylbenzene include: (1) absorption; (2) metabolism to active metabolite; (3) distribution of active metabolite to kidney; (4) detoxification/elimination of active metabolite; (5) exacerbation of CPN; and (6) promotion of kidney tumors. Based upon the weight of evidence described above, the proposed MOA for male rat kidney tumors satisfies the modified Hill criteria for causation for ethylbenzene-induced kidney tumors. Because of critical qualitative, and to a certain extent, quantitative species differences, this MOA is not expected to be relevant to human health. Therefore, rat kidney tumors are not a suitable basis for risk assessment, and implications of the MOA to an appropriate internal dose measure and the nature of the dose-response relationship are not required. Consideration for an alternative MOA (direct genotoxicity) is provided in **Section 8.3.3.5**.

8.3.3.2 Proposed MOA for Liver Tumors

Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

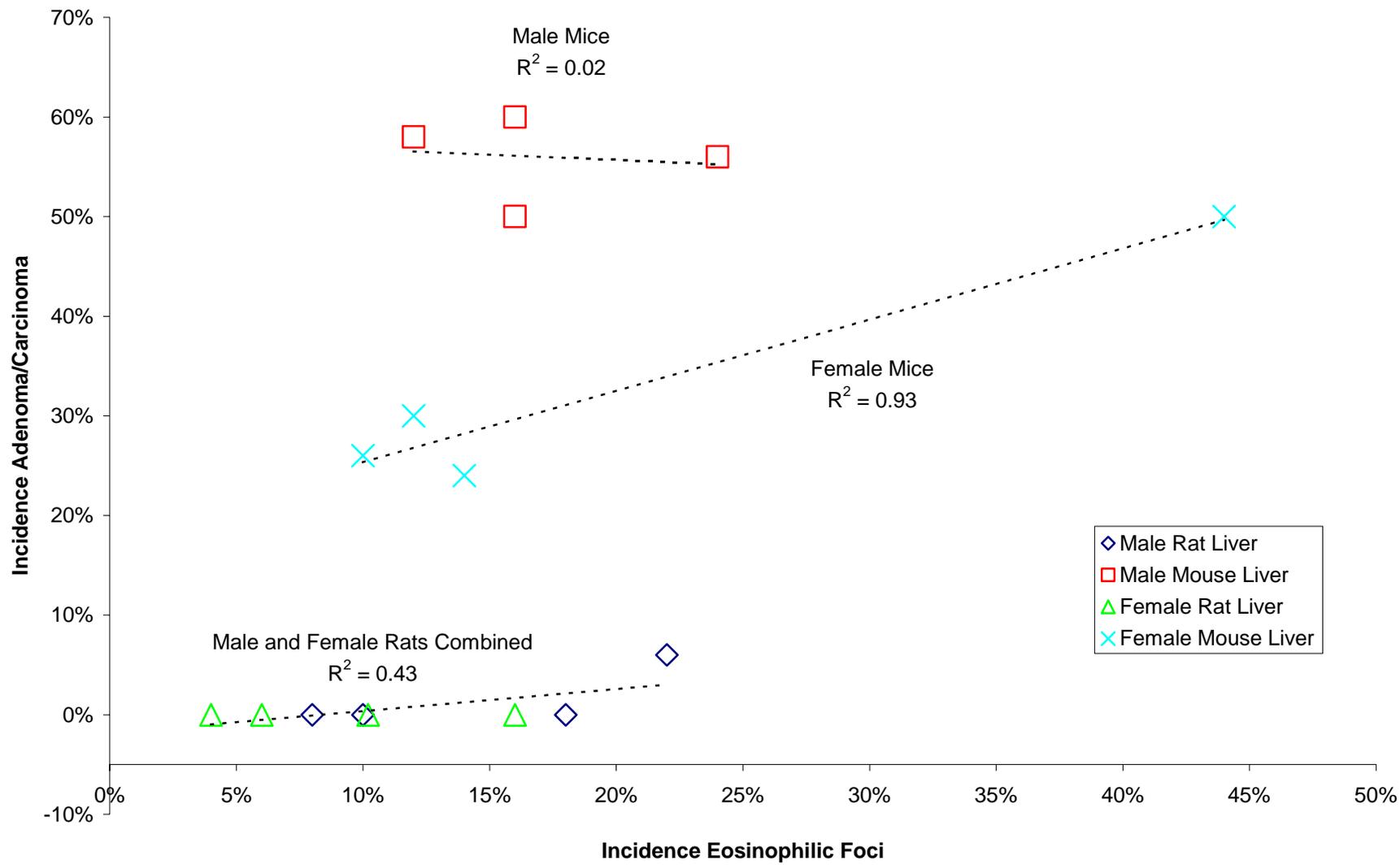
Ethylbenzene has been shown to cause liver tumors in female mice following chronic inhalation exposures. It is hypothesized that ethylbenzene produces liver tumors in female mice by a nongenotoxic MOA by inducing hepatic enzyme activity, which results in an increase in cell proliferation ultimately leading to promotion of liver tumors. The key events in the MOA include: (1) absorption; (2) distribution of parent chemical to liver; (3) phenobarbital-like enzyme induction; (4) detoxification/elimination of parent chemical; (5) increased cell proliferation, inhibition of apoptosis, hypertrophy, development of altered hepatic foci; and (6) promotion of liver tumors. The weight of evidence supporting this MOA is evaluated using the modified Hill criteria below.

- *Strength of Association* – Following chronic exposure to ethylbenzene, female mice have shown an increased incidence of liver tumors (NTP, 1999; Chan *et al.*, 1998) and increased liver weights (Stott *et al.*, 2003; NTP, 1992a; Cragg *et al.*, 1989), indicative of treatment-related toxicity. A strong correlation is observed between the incidence of eosinophilic foci and liver tumors in female mice (**Figure 8-3**), however, this association appears to be unique to female mice since it is not observed in male mice (who have a similar background rate of eosinophilic foci, but a much higher background rate of liver tumors), or in rats of either sex. A primary metabolite of ethylbenzene in the liver is 1-phenylethanol (Engstrom, 1984; Saghir *et al.*, 2006). The primary cytochrome P450 isozyme responsible for this reaction is the CYP2E1 (Imaoka and Funae, 1991; Sequeira *et al.*, 1992, Yuan *et al.*, 1997a,b; Sams *et al.*, 2004). In rats, metabolic saturation and induction of CYP2E1 is found, as well as induction of CYP2B1 and 2B2

(Imaoka and Funae, 1991; Sequeira *et al.*, 1992, Yuan *et al.*, 1997a,b). These observations are indicative of a phenobarbital-type liver response (CYP2B1-specific enzyme induction and hepatocellular proliferation, eosinophilic foci) (Bus, 2006). Chronic induction of P450 isozymes has been associated with liver tumors in rodents (Grasso and Hinton, 1991). Eosinophilic foci are considered to be a precursor to liver tumors (NTP, 1999; Chan *et al.*, 1998). Increased liver weights and liver tumors associated with eosinophilic foci are characteristic of a phenobarbital-type liver response (Dalton *et al.*, 2003). In addition, the Phenobarbital-type liver tumor MOA only applies to nongenotoxic compounds, entirely consistent with the negative genotoxic profile of ethylbenzene. Therefore, data are available to support this MOA. The incidence of thyroid gland follicular cell hyperplasia was significantly increased in male and female mice exposed to 750 ppm ethylbenzene (NTP, 1999), an effect that is also consistent with a phenobarbital-like alternation in thyroid hormone clearance (Meek *et al.*, 2003).

- *Consistency of Association* – Ethylbenzene has been shown to enhance liver tumors in mice (NTP, 1999; Chan *et al.*, 1998) and cause an increase in liver weights in female mice (Stott *et al.*, 2003; NTP, 1992a; Cragg *et al.*, 1989). Various studies have shown that ethylbenzene induces CYP2E1, 2B1, and 2B2 in rats (Imaoka and Funae, 1991; Sequeira *et al.*, 1992, Yuan *et al.*, 1997a,b; Sams *et al.*, 2004). The metabolite of ethylbenzene, 1-phenylethanol, which does not induce cytochrome P450 enzymes, did not produce an increase in liver tumors in chronically exposed mice (NTP, 1990). Therefore, data are available to support this MOA.
- *Specificity of Association* – It appears that the target organ of ethylbenzene effects in female mice is the liver, whether it is tumor formation or increased liver weight (NTP, 1999; Chan *et al.*, 1998; Stott *et al.*, 2003; NTP, 1992a; Cragg *et al.*, 1989). No other tumor types have been reported for female mice following exposure to ethylbenzene. On the other hand, liver tumors were absent in male mice in chronic exposures to ethylbenzene, and in rats of both sexes, even though liver weights are increased by exposure to ethylbenzene (NTP, 1992a, 1999; Chan *et al.*, 1998; Stott *et al.*, 2003). It is not understood why tumors are only increased in female mice. However, the magnitude of the increase in female mice was relatively weak, suggesting that the effects of ethylbenzene on the underlying processes in the MOA, may also be weak. Although organ weight changes do not demonstrate sex- and species-specificity, the incidence of liver tumors was only increased in the sex and species in which eosinophilic foci were increased (female mice exposed to the highest concentration), and therefore, this MOA explains some of the species and sex-specificity of observations made for liver tumors.

Figure 8-3. Correlation Plot for Liver Lesions in Rats and Mice Exposed to Ethylbenzene



- *Dose-Response Concordance* – Following chronic inhalation exposure to 750 ppm of ethylbenzene, an increased incidence of female mouse liver tumors was observed, as well as an increase incidence of eosinophilic foci (NTP, 1999; Chan *et al.*, 1998). In a 13-week, subchronic inhalation study, mice exposed to 750 and 1000 ppm experienced an increase in liver weights (NTP, 1992a). Also, Stott *et al.* (2003) reported an increase in liver weights and enzyme activities in mice exposed to 750 ppm for either one or four weeks. Inspection of the incidence data for eosinophilic foci and liver tumor data in female mice reveals that their dose-response behaviors are nearly identical in showing a significant increase only at the highest concentration. Furthermore, tumors were only increased in animals exposed to 750 ppm ethylbenzene, which is the dose known to be above metabolic saturation (Charest-Tardif *et al.*, 2006), whereas no increase in tumors was observed at 250 ppm, which is below metabolic saturation (*i.e.*, an exposure level not associated with compensatory enzyme induction is not associated with tumors). Therefore, this MOA provides concordance with the dose-response data for liver tumors.
- *Temporal Relationship* – After two years exposure to ethylbenzene, liver tumor incidences in female mice were increased (NTP, 1999; Chan *et al.*, 1998). Liver tumors were late developing (*i.e.*, first incidence observed on days 562-659 in treated animal groups compared to day 565 in control animals), which is consistent with a promotional MOA involving enzyme induction. Increases in enzyme activity in the liver have been shown as early as one week of ethylbenzene inhalation exposure (Stott *et al.*, 2003). Similarly, liver weight increases have been reported as early as one week of ethylbenzene exposure (Stott *et al.*, 2003). Therefore, this MOA is consistent with the temporal relationship in that the underlying events in the MOA can occur well before the appearance of liver tumors.
- *Biological Plausibility and Coherence* – Liver enzyme induction followed by cell proliferation is a biologically plausible MOA for induction of female mouse liver tumors. Similar to the ethylbenzene-induced mouse liver tumor response, the toxicity profile of a phenobarbital-type response is also specifically associated with increased female mouse liver tumors (Bus, 2006). The critical role of the liver physiologic and metabolic adaptive responses to high-dose ethylbenzene exposure in mediating the female mouse liver tumor response is further supported by findings from chronic oral studies of the ethylbenzene metabolite, 1-phenylethanol, in mice (NTP, 1990). Treatment of both sexes of B6C3F1 mice with doses up to 750 mg/kg-day, 5 days/week for two years produced no evidence of liver tumors or changes in hepatocellular histopathology. Since direct administration of 1-phenylethanol does not produce phenobarbital-like metabolic and physiologic adaptive responses, the absence of liver tumors observed in mice exposed to 1-phenylethanol is consistent with the MOA proposed for ethylbenzene. Taken together, these findings provide strong support that the MOA of ethylbenzene-induced female mouse liver tumors are secondary to a phenobarbital-like enzyme induction and cell proliferation.

Are Key Events in the Animal MOA Plausible in Humans?

The key events for ethylbenzene producing liver tumors in mice are presented in **Table 8-6**. The key events for ethylbenzene-induced mouse liver tumors are the same as those reported for Oxazepam, Pyrethrins and fenbuconazole, chemicals for which a phenobarbital-type mechanism of action have been proposed (Cunningham *et al.*, 1994; Griffin *et al.*, 1996; Parkinson *et al.*, 2006; Juberg *et al.*, 2006; Price *et al.*, 2007). Qualitative differences between female mice and other species include: (1) an increase in altered hepatic foci (eosinophilic) was observed in female mice, but not in male mice or in rats of either sex; and (2) human experience with chronic exposure to phenobarbital indicates that this MOA is not relevant to humans (Whysner *et al.*, 1996; Holsapple *et al.*, 2006).

Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans?

This question is moot because the weight of evidence for the postulated mode of action for liver tumors in animals exposed to ethylbenzene is not relevant in humans due to qualitative species differences. However, quantitative differences do exist between mice and humans and include: (1) rodent exposures are orders of magnitude higher than expected human exposure; (2) liver tumor responses were limited to the high, enzyme-inducing exposure only (750 ppm), a concentration well above non-linear pharmacokinetic behavior; (3) rates of metabolism in liver and lung microsomes are higher in mice than in humans (Saghir *et al.*, 2006); and (4) background rates for liver tumors is higher in female mice (~26%) compared to humans (~0.6%, SEER, 2006).

Conclusion

Confidence in the proposed MOA is high since it is well supported by available studies. Following exposure to ethylbenzene, the key events in the MOA include: (1) absorption; (2) distribution of parent chemical to liver; (3) phenobarbital-like enzyme induction; (4) detoxification/elimination of parent chemical; (5) increased cell proliferation, inhibition of apoptosis, hypertrophy, development of altered hepatic foci; and (6) promotion of liver tumors. The proposed MOA is well supported and meets the modified Hill criteria for causation for liver tumors female mice. Confidence in the proposed MOA is high, since it is supported by observations (altered foci) made in the key study (NTP, 1999), as well as for structurally similar chemicals (oxazepam, pyrethrins and fenbuconazole) that act via a phenobarbital-type MOA (Cunningham *et al.*, 1994; Griffin *et al.*, 1996; Parkinson *et al.*, 2006; Juberg *et al.*, 2006; Price *et al.*, 2007). Because phenobarbital-type liver responses have been deemed not relevant to humans due to qualitative, as well as quantitative, species differences (Whysner *et al.*, 1996; Holsapple *et al.*, 2006), selection of an appropriate internal dose measure and low-dose extrapolation is not required. Consideration for an alternative MOA (direct genotoxicity) is provided in **Section 8.3.3.5**.

Table 8-6. Key Events in the MOA for Ethylbenzene-Induced Liver Tumors

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Exposure	Exposures to EB occur under controlled conditions in a laboratory setting	Exposures to EB can occur at the workplace, from consumer products, and from the environment	Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals	Rodent exposures are orders of magnitude higher than expected human exposure	None identified. Linearity is assumed
Absorption	EB is well absorbed following inhalation (Chin <i>et al.</i> , 1980) or ingestion (El Mastri <i>et al.</i> , 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsurata, 1982; Morgan <i>et al.</i> , 1991; Susten <i>et al.</i> , 1990).	EB is well absorbed following inhalation (Bardodej and Bardodejova, 1970; Engström and Bjurström, 1978; Åstrand <i>et al.</i> , 1978; Gromiec and Piotrowski 1984) and dermal (Dutkiewicz and Tyras, 1967) routes. EB is assumed to be well absorbed via ingestion.		None identified	None identified. Linearity is assumed
Distribution of parent chemical to liver	Absorbed EB is rapidly distributed to all tissues in the body (Chin <i>et al.</i> , 1980; Engström <i>et al.</i> , 1985; Cappaert, 2000)	Rapid distribution of EB to human tissues is assumed.	None identified. The process dictating absorption are assumed to qualitatively similar.	None identified. The process dictating absorption are assumed to quantitatively similar.	None identified. Linearity is assumed

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Phenobarbital-like enzyme induction	In rats, induction of CYP2E1, CYP2B1 and CYP2B2 has been reported (Imaoka and Funae, 1991; Sequeira <i>et al.</i> , 1992, Yuan <i>et al.</i> , 1997a,b).	Enzyme induction is assumed	Because human experience with chronic high-dose exposures to Phenobarbital is not associated with liver tumor formation, phenobarbital-type liver tumor responses have been deemed not relevant to humans (Holsapple <i>et al.</i> , 2006).	None identified.	Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation)
Detoxification/Elimination of parent chemical	In rats, about 83% of EB was excreted in the urine over 72 hours; ~8% was exhaled; ~0.7% was excreted in the feces; ~0.03% in exhaled CO ₂ ; ~8.2% in expired gases; ~0.2% remained in the tissues (Chin <i>et al.</i> , 1980).	Absorbed EB is excreted in the urine and exhaled breath (Bardodej and Bardodejova, 1970; Åstrand <i>et al.</i> , 1978; Engström <i>et al.</i> , 1984; Gromiec and Piotrowski, 1984; Kawai <i>et al.</i> , 1992; Knecht <i>et al.</i> , 2000)	None identified. The process dictating elimination are assumed to qualitatively similar.	Species differences in urinary metabolites reflect differences in metabolism	Enzyme induction and metabolic saturation above concentration of 500 ppm

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Increased cell proliferation, inhibition of apoptosis, hypertrophy, development of altered hepatic foci	Eosinophilic foci are considered to be a precursor to liver tumors (NTP, 1999; Chan <i>et al.</i> , 1998). Increased liver weights and liver tumors associated with eosinophilic foci are characteristic of a phenobarbital-type liver response (Dalton <i>et al.</i> , 2003)	No evidence	An increase in altered hepatic foci (eosinophilic) was observed only in female mice, but not in male mice or in rats of either sex, and is not expected to occur in humans.	None identified.	Threshold events are possible
Promotion of liver tumors				Human experience with phenobarbital indicates that this MOA is not relevant to humans (Holsapple <i>et al.</i> , 2006). Background rates for liver tumors is as follows: male mouse (~58%) > female mouse (~26%) > human (~0.6%, SEER, 2006) ≈ male rat (~0%) ≈ female rat (~0%)	Threshold events are possible

8.3.3.3 Proposed Lung Tumor MOA

Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

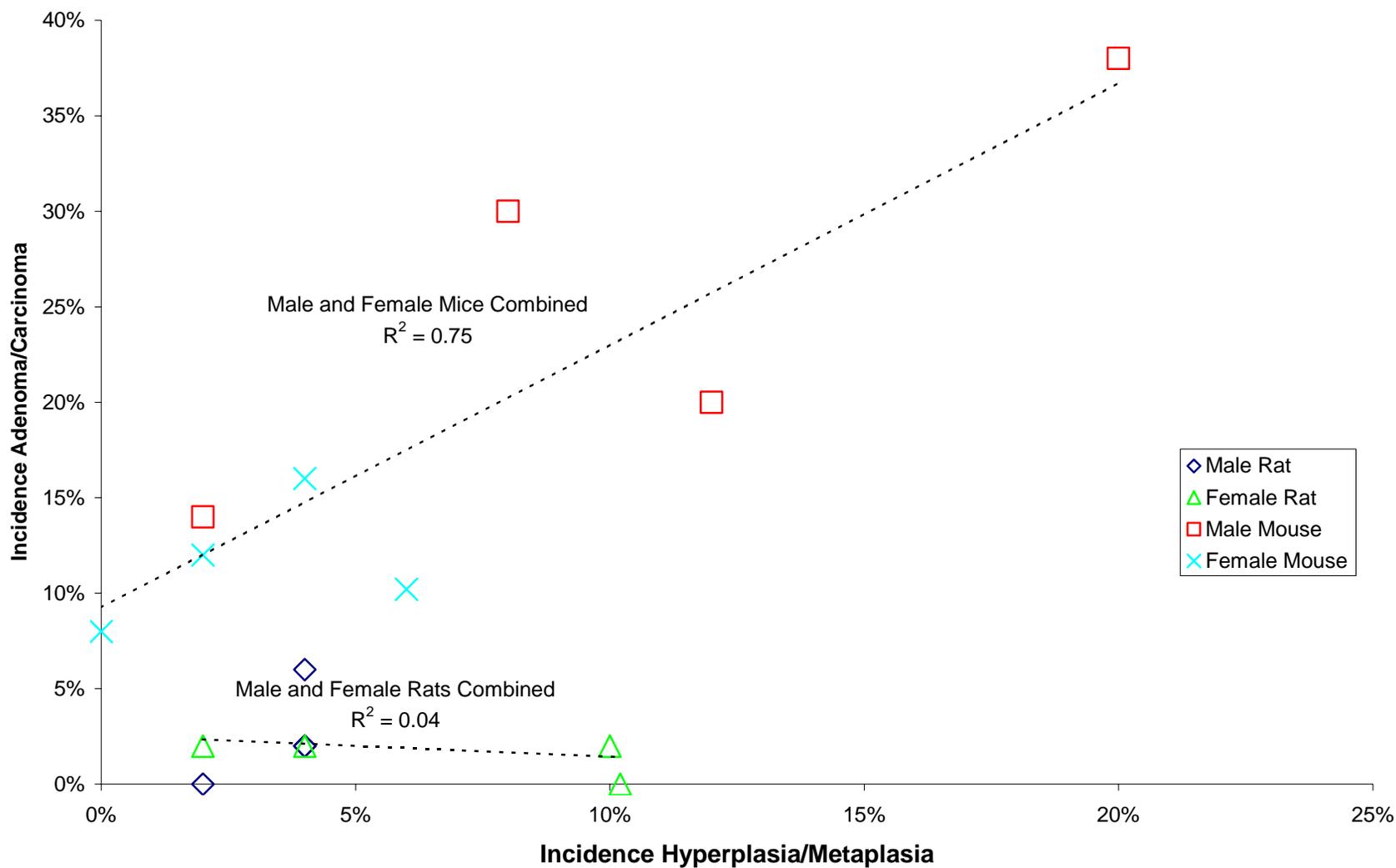
Male mice have been reported to have an increased occurrence of lung tumors following chronic exposure to ethylbenzene. The key events in the proposed MOA include (1) absorption; (2) distribution of ethylbenzene to lung; (3) metabolism to active metabolite; (4) detoxification/ elimination of active metabolite; (5) possible oxidative stress secondary to high-dose GSH depletion and/or high-dose mediated CYP450 ethylbenzene metabolism; (6) arylation of macromolecules leading to cytotoxicity when detoxification and repair capacities are exceeded; and (7) promotion/progression of lung tumors. By analogy to the ethylbenzene structural analog styrene, the hypothesized MOA for ethylbenzene -induced lung tumors involves the formation of ring-oxidized metabolites of ethylbenzene to cytotoxic metabolites by CYP2F2, which is expressed at relatively high levels in mouse lung (Cruzan *et al.*, 2002). Ethylbenzene is metabolized to 4-ethylphenol and 2-ethylphenol following exposure (**Figure 8-1**). These metabolites are further metabolized to catechols and hydroquinones, which then undergo additional auto-oxidation to reactive, cytotoxic quinone metabolites capable of binding to cellular macromolecules, likely derived from catechol and/or hydroquinone intermediates. At sufficiently high exposures, the formation of such metabolites can also deplete intracellular GSH, enhancing the ability to attack other critical cellular macromolecules (*e.g.*, nucleophilic sites on proteins) and resulting in ensuing cell cytotoxicity (this mechanism is consistent with existing data with structurally related chemicals, styrene and naphthalene). As observed by Stott *et al.* (2003), the cytotoxicity results in chronic cell proliferation leading to the late-developing tumors. The weight of evidence for the proposed MOA is evaluated below using the modified Hill criteria.

- *Strength of Association* – Chronic inhalation exposure of ethylbenzene has shown to increase the incidence of lung tumors among male mice (NTP, 1999; Chan *et al.*, 1998). However, the magnitude of the increase in male mice is relatively weak, suggesting that the effects of ethylbenzene on the underlying processes in the MOA, may also be weak. A good correlation is observed between the incidence of lung hyperplasia/metaplasia and the incidence of lung tumors in mice (**Figure 8-4**). This association appears to be specific to mice, with males demonstrating a greater response than females, but is lacking in rats of both sexes. Mouse lung tumors may arise through a mouse Clara cell specific CYP450 2F2 metabolite (Bus, 2006), analogous to styrene lung toxicity MOA studies (Cruzan *et al.*, 2002). These metabolites of ethylbenzene include 4-ethylphenol and 2-ethylphenol that are further metabolized to catechols, hydroquinones, and downstream quinone metabolites (Engstrom, 1984; Midorikawa *et al.*, 2004; Saghir *et al.*, 2006). Metabolites of catechol and hydroquinone have been shown to autooxidize to protein-reactive and cytotoxic quinones (Rossi *et al.*, 1986; Gant *et al.*, 1988; O'Brien, 1991; Tapper *et al.*, 2000; Bus, 2006). In a recent study, 4-ethylcatechol and ethylhydroquinone, which are metabolized from 4-ethylphenol and 2-ethylphenol, respectively, have been shown to induce the formation of 8-oxo-dG in calf thymus DNA at high concentrations (Midorikawa *et al.*, 2004). As

indicated above, oxidative damage may arise through depletion of cellular GSH or via cytochrome P450 metabolism. Additional evidence supporting the formation of reactive metabolites include the observation that mouse and rat lung microsomes (and to a lesser extent, mouse liver microsomes) exhibited decreasing amounts of ring-oxidized metabolite formation with increasing concentrations of ethylbenzene. This suggests the possibility of cytochrome P450 suicide inhibition by reactive ring-oxidized metabolite(s) (quinones) (Saghir *et al.*, 2007). This inhibition appears to be isozyme-specific (CYP2F2) in that generation of alkyl-oxidized metabolites (CYP2E1) was not similarly decreased with increasing ethylbenzene substrate concentrations. Therefore, data are available to support this MOA.

- *Consistency of Association* – Various studies have shown an increase in male mouse lung tumors (NTP, 1999; Chan *et al.*, 1998). Also, a short-term inhalation study of ethylbenzene has shown alterations in lung cell populations (Stott *et al.*, 2003). The metabolism of ethylbenzene has been studied extensively in rats with results showing trace amounts of 4-ethylphenol and 2-ethylphenol being produced (Engstrom, 1984). Additionally, ethylbenzene has been shown to produce 4-ethylphenol and 2-ethylphenol in mouse lung microsomes (Saghir *et al.*, 2006). These metabolites are further metabolized to catechols and hydroquinones. Therefore, data are available to support the consistency of this MOA. Alkyl oxidation of ethylbenzene, the primary metabolic route of ethylbenzene metabolism, is likely not responsible for lung tumorigenicity in that the NTP bioassay of 1-phenylethanol indicates that this major metabolite is not lung toxic or tumorigenic. Data for a structurally similar chemical, styrene, also support the formation of ring-oxidized metabolites rather than alkyl-oxidized metabolites in producing lung toxicity. Specifically, lung toxicity was similar in CYP2E1 knockout and wild-type mice, indicating that alkyl oxidation (CYP2E1 activity) does not correlate with lung, whereas ring oxidation (CYP2F2 activity), which is similar in wild-type and knockout mice, does correlate with lung toxicity (Carlson, 2004). This points strongly to ring oxidized metabolite(s) as the drivers for lung tumorigenicity.
- *Specificity of Association* – The main target organs of ethylbenzene in male mice are the lungs, including increased tumor formation and alterations in cell populations (NTP, 1999; Chan *et al.*, 1998, Stott *et al.*, 2003). Cytotoxicity is localized to lung sites (terminal bronchioles, Clara cells) that are known to be enriched in specific P450 (2F2) likely to contribute to ring oxidation of ethylbenzene. Because the incidence of hyperplasia/metaplasia was increased in the only sex and species (male mice) in which tumors were increased (**Table 8-3**), this MOA is consistent with the species and sex-specific observations made for lung tumors in rodents.

Figure 8-4. Correlation Plot for Lung Lesions in Rats and Mice Exposed to Ethylbenzene



- Dose-Response Concordance* - Following chronic inhalation exposure to 750 ppm of ethylbenzene, but not at lower concentrations of 75-250 ppm, an increased incidence of lung tumors was observed in male mice (NTP, 1999; Chan *et al.*, 1998). Also, Stott *et al.* (2003) reported evidence of alterations in cell populations in the lungs of mice exposed to 750 ppm for either one or four weeks. Mouse lung microsomes exposed to either 750 or 7500 ppm of ethylbenzene produced 4-ethylphenol and 2-ethylphenol (Saghir *et al.*, 2006). These metabolites are further metabolized to catechols and hydroquinones. Metabolites of catechol and hydroquinone have been shown to autooxidize to protein-reactive and cytotoxic quinones capable of depleting cellular GSH levels (Rossi *et al.*, 1986; Gant *et al.*, 1988; O'Brien, 1991; Tapper *et al.*, 2000; Bus, 2006), and also have been proposed to go through oxidative redox cycling, possibly resulting in intracellular oxidative stress (Irons *et al.*, 1981; Greenlee *et al.*, 1981), although redox cycling does not appear to be important for mono-substituted benzenes. Therefore, data are available to support the dose-response concordance for this MOA.
- Temporal Relationship* – Lung tumors in male mice have been shown in a chronic two-year inhalation study (NTP, 1999; Chan *et al.*, 1998). Effects of ethylbenzene on the lung, however, have been reported following much shorter exposures. Ethylbenzene has been shown to alter cell populations in the lungs of mice as early as one week of exposure (Stott *et al.*, 2003). Mouse lung microsomes exposed to ethylbenzene for 30 minutes resulted in the formation of 4-ethylphenol and 2-ethylphenol (Saghir *et al.*, 2006), which in turn could result in the further metabolism to catechols and hydroquinones. With respect to a structurally similar chemicals (styrene, naphthalene), glutathione depletion, oxidative stress, and cytotoxicity has been observed in mouse Clara cells following acute exposures and in *in vitro* studies (Plopper *et al.*, 2001; Harvilchuck and Carlson, 2006; Phimister *et al.*, 2005). Therefore, this MOA is consistent with the temporal relationship in that underlying effects in the MOA are observed well before the appearance of lung tumors in male mice.
- Biological Plausibility and Coherence* –Ring-oxidation of ethylbenzene to ring-oxidized metabolites appears to be a biologically plausible MOA for male mouse lung tumors. However, CYP450 2F2 is a Clara cell specific enzyme involved in ethylbenzene metabolism and is present at high levels in mouse lung. Also, human lungs contain far fewer numbers of Clara cells than mice (Stott *et al.*, 2003), and human lung microsomes failed to or marginally metabolize ethylbenzene (Saghir *et al.*, 2006). This observation is consistent with results obtained previously for a structurally similar chemical, styrene and coumarin, in which metabolism is high in mouse respiratory tissue, but could not be detected in human respiratory tissue (Green *et al.*, 2001; Vassallo *et al.*, 2004). In addition, the human lung differs markedly from the mouse lung in the number and morphology of its Clara cells, which make humans less sensitive than mice to toxicity due to reactive metabolites (Green, 2000). The consistency of the relative species differences across chemicals with similar modes of action suggests that

the decreased activity in human respiratory tract is not due to viability issues associated with human tissues, but instead reflects a fundamental species difference with respect to the distribution, expression, and/or activity of CYP2F in the respiratory tract. Styrene, a structural analog of ethylbenzene, has been reported to increase the incidence of mouse lung tumors (Cruzan *et al.*, 2002). The proposed MOA for lung tumors following styrene exposure is mediated by metabolites formed through the CYP2F2 enzyme (Cruzan *et al.*, 2002), as is supported by reports of similar pulmonary toxicity in wild-type and CYP2E1 knockout mice, which demonstrate the absence of a role for CYP2E1 metabolites (alkyl oxidation) in mouse pulmonary effects (Carlson, 2004). Additionally, benzene and naphthalene, which are structurally similar to ethylbenzene, has been shown to induce mouse lung tumors following exposure via the formation of metabolites; however, the metabolites differ from those generated by the metabolism of ethylbenzene (NTP, 1986, 1992b). Likewise, inhalation exposure to naphthalene resulted in an increased incidence of lung tumors in female mice (NTP, 1992b). In a review by Gram (1997), naphthalene is reported as being metabolized via CYP2F2 enzyme in mouse lung. In addition, the metabolites formed are reported as being cytotoxic to Clara cells in mouse lung (Gram, 1997). Therefore, this MOA is plausible and coherent.

Are Key Events in the Animal MOA Plausible in Humans?

The key events for ethylbenzene producing lung tumors in mice are presented in **Table 8-7**. Currently no qualitative differences between mice and humans have been identified. The key events for the MOA are thus assumed to be qualitatively similar between these two species. However, the possibility remains that important qualitative differences, with respect to either CYP2F (CYP2F2 in mice; CYP2F1 in humans) expression or activity, exists between humans and mice. For this current assessment, differences between CYP2F expression or activity are assumed to be quantitative in nature (see next section).

Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans?

Given that the qualitative impacts of the proposed MOA on tumor outcomes is not fully defined, quantitative differences between mice and humans must also be considered and include: (1) Rodent exposures are orders of magnitude higher than expected human exposure; (2) Mouse lung has a larger fraction than the human lung with respect to Clara cells (Green, 2000), which are particularly sensitive (3) Rates of metabolism for ethylbenzene in lung microsomes exhibit clear species differences, with rates in mice being greater than the corresponding rates in humans (Saghir *et al.*, 2006), an observation that is consistent with reports for chemicals (styrene, naphthalene, coumarin) with a similar mode of action (Green *et al.*, 2001; Vassallo *et al.*, 2004); and (4) Background rates for lung tumors are higher in male mice (~14%) than in humans (~7%, SEER, 2006). Given these species differences, the MOA is assumed to be plausible in humans, but humans are expected to be much less sensitive than mice to the pulmonary effects of ethylbenzene.

Table 8-7. Key Events in the MOA for Ethylbenzene-Induced Lung Tumors

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Exposure	Exposures to EB occur under controlled conditions in a laboratory setting	Exposures to EB can occur at the workplace, from consumer products, and from the environment	Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals	Rodent exposures are orders of magnitude higher than expected human exposure	None identified. Linearity is assumed
Absorption	EB is well absorbed following inhalation (Chin <i>et al.</i> , 1980) or ingestion (El Mastri <i>et al.</i> , 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsurata, 1982; Morgan <i>et al.</i> , 1991; Susten <i>et al.</i> , 1990).	EB is well absorbed following inhalation (Bardodej and Bardodejova, 1970; Engström and Bjurström, 1978; Åstrand <i>et al.</i> , 1978; Gromiec and Piotrowski 1984). and dermal (Dutkiewicz and Tyras, 1967) routes. EB is assumed to be well absorbed via ingestion.	None identified. The processes dictating absorption are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar.	None identified. Linearity is assumed
Distribution of ethylbenzene to lung	Absorbed EB is rapidly distributed to all tissues including the lung in animals exposed via inhalation (Chin <i>et al.</i> , 1980; Cappaert, 2000) and dermal routes (Susten <i>et al.</i> 1990). Rapid distribution following ingestion is assumed.	Rapid distribution to all tissues is assumed.	None identified. The processes dictating distribution are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar	None identified. Linearity is assumed

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Metabolism to active metabolite	Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of EB (McMahon and Sullivan, 1966, 1968; Engström, 1984; Kaubisch <i>et al.</i> , 1972 Stott <i>et al.</i> , 2003; Saghir <i>et al.</i> , 2006)	Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of EB (Engström <i>et al.</i> , 1984; Sams <i>et al.</i> , 2004; Saghir <i>et al.</i> , 2006)	None identified. The processes dictating metabolism are assumed to be qualitatively similar.	Rates of metabolism of EB in lung microsomes exhibit clear species differences: mice > rats ≥ humans (Saghir <i>et al.</i> , 2006)	Enzyme induction and metabolic saturation achieved above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation)
Detoxification/ Elimination of active metabolite	In rats, about 83% of EB was excreted in the urine over 72 hours; ~8% was exhaled; ~0.7% was excreted in the feces; ~0.03% in exhaled CO ₂ ; ~8.2% in expired gases; ~0.2% remained in the tissues (Chin <i>et al.</i> , 1980).	Absorbed EB is excreted in the urine and exhaled breath (Bardodej and Bardodejova, 1970; Åstrand <i>et al.</i> , 1978; Engström <i>et al.</i> , 1984; Gromiec and Piotrowski, 1984; Kawai <i>et al.</i> , 1992; Knecht <i>et al.</i> , 2000)	None identified. The process dictating elimination are assumed to qualitatively similar.	Differences in urinary metabolites reflect differences in metabolism	None identified. Linearity is assumed
Oxidative stress secondary to high-dose GSH depletion and/or high-dose mediated CYP450 ethylbenzene metabolism	Evidence in rodents is inferred from structurally similar chemicals (styrene)	No evidence	None identified	None identified	Thresholds associated with depletion of tissue antioxidants

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Oxidation of macromolecules leading to cytotoxicity	Evidence in rodents is inferred from structurally similar chemicals (styrene)	No evidence	None identified	None identified	None identified. Linearity is assumed
Promotion/Progression of lung tumors	Evidence in rodents is inferred from structurally similar chemicals (styrene)	No evidence	None identified	Background rates for lung tumors are as follows: male mouse (~ 14%) > female mouse (~8%) ≈ human (~7%, SEER, 2006) ≈ male rat (~6%) > female rat (~2%)	None identified. Linearity is assumed

Conclusion

Following exposure to ethylbenzene, the key events in the proposed MOA include (1) absorption; (2) distribution of ethylbenzene to lung; (3) metabolism to active metabolite; (4) detoxification/ elimination of active metabolite; (5) possible oxidative stress secondary to high-dose GSH depletion and/or high-dose mediated CYP450 ethylbenzene metabolism; (6) arylation of macromolecules leading to cytotoxicity when detoxification and repair capacities are exceeded; and (7) promotion/progression of lung tumors. The proposed MOA satisfies the modified Hill criteria for causation for lung tumors. Confidence in the proposed MOA is considered to be medium since support for this MOA largely comes from information collected from structurally similar chemicals rather than ethylbenzene itself. Consideration for an alternative MOA (direct genotoxicity) is provided in **Section 8.3.3.5**.

Based upon the proposed MOA for ethylbenzene in producing mouse lung tumors, inferences can be made regarding the internal dose and method for low-dose extrapolation used in the dose-response assessment. With respect to internal dose, the concentration of catechol and quinone metabolites in target tissue is expected to be proportionate to tissue tumor response. Because the PBPK model does not provide descriptions for individual metabolites, the total amount metabolized/kg tissue-week would serve as a useful internal dose surrogate. With respect to low dose extrapolation, the proposed MOA suggests that doses below a toxic threshold would not be expected to result in tumor formation and thus, a nonlinear method of extrapolation (RfD approach) is indicated.

8.3.3.4 Proposed Leydig Cell Tumor MOA

Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

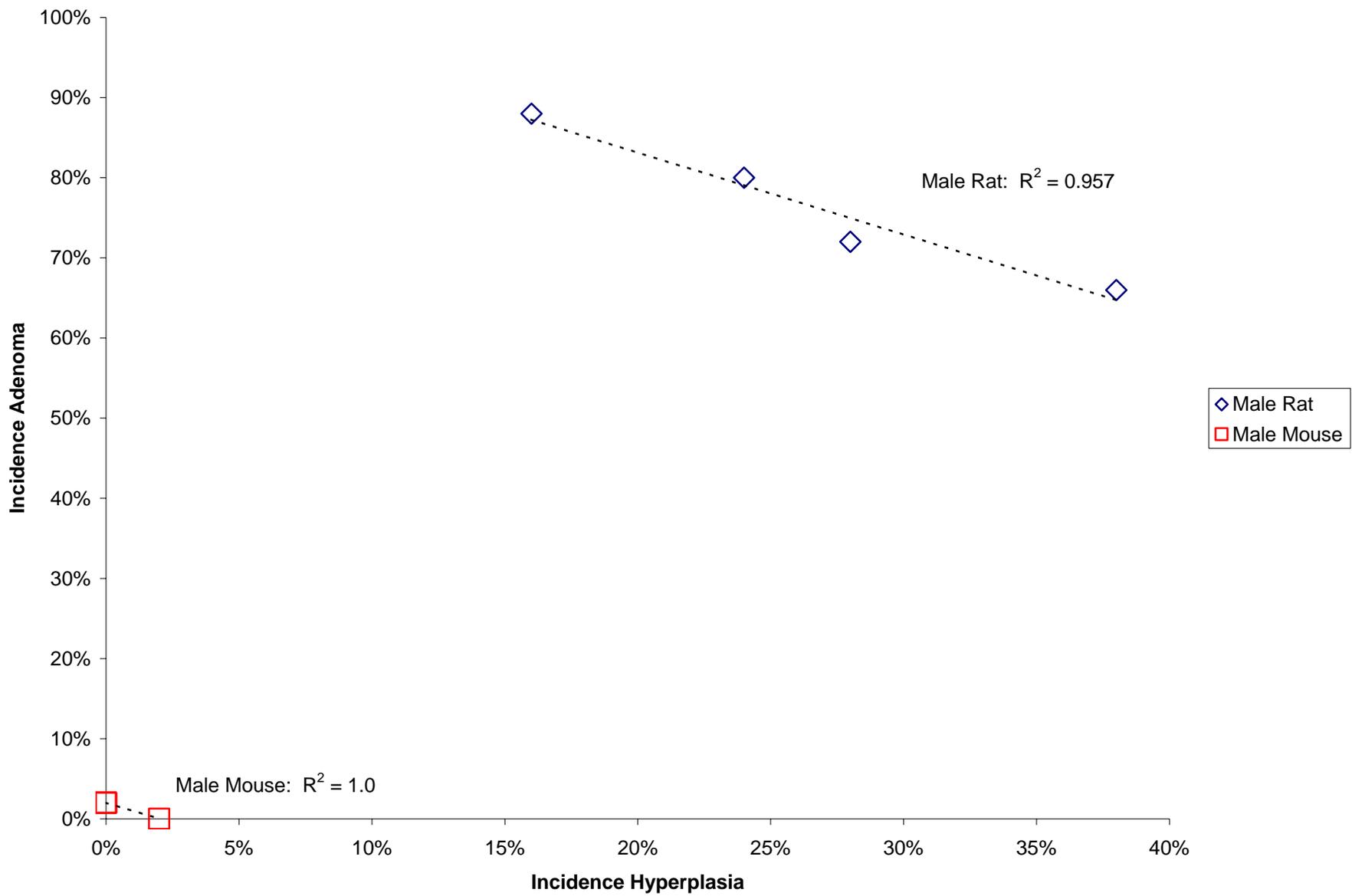
Male rats have been reported to have an increased occurrence of LCT following chronic exposure to ethylbenzene. The key events in the proposed MOA include (1) absorption; (2) distribution of ethylbenzene to the liver; (3) enzyme induction and increased testosterone clearance; (4) perturbation serum testosterone levels; and (5) promotion/progression of LCT. The weight of evidence for the proposed MOA is evaluated below using the modified Hill criteria.

- *Strength of Association* – The association between ethylbenzene exposure and LCTs (all adenomas, no carcinomas) in male rats is relatively weak. Although a significant increase in the unadjusted incidence of LCTs male was observed in rats exposed to 750 ppm (NTP, 1999), the dose-response relationship is nonmonotonic, exhibiting a slight decrease in incidence at the lowest concentration before increasing at the middle and high concentrations (**Table 8-3**). More importantly, both the survival-adjusted and terminal incidence rates for this tumor are 100% for all animals (treated and untreated). A negative correlation is observed between Leydig cell hyperplasia and LCT incidence (**Figure 8-5**). This negative correlation is possibly explained by the fact that the

incidence of having either Leydig cell hyperplasia or adenoma is approximately 100% in exposed and unexposed animals, and that the difference between these two lesions is the size of the nodule (adenoma classification is designated when the diameter exceeds either one or three seminiferous tubule cross-sections) (Clegg *et al.*, 1997). Therefore as lesions progress from hyperplasia to adenoma, the incidence of hyperplasia would be expected to decrease as the incidence of adenoma increases.

- *Consistency of Association* – The weak association between ethylbenzene exposure and LCTs is limited to a single study in rats (NTP, 1999). No testicular lesions were observed in rats or mice exposed to ethylbenzene for 13 weeks (NTP, 1992).
- *Specificity of Association* – The weak association between ethylbenzene exposure and LCTs is specific to rats since these tumors were not increased in similarly exposed mice (NTP, 1999).
- *Dose-Response Concordance* – Because a negative correlation was reported between the incidence of hyperplasia and LCT in male rats, and because subchronic exposures to concentrations up to 1,000 ppm failed to produce testicular effects in rats (NTP, 1992) dose-response concordance is not supported for this mode of action.
- *Temporal Relationship* – Induction of cytochrome P450 isozymes and increased testosterone hydroxylation have been observed in rats within 24-48 hours after exposure to ethylbenzene (Yuan *et al.*, 1997), however circulating levels of testosterone were not determined. Perhaps more importantly, no testicular effects were noted in rats and mice exposed to 0, 100, 250, 500, 750, or 1,000 ppm for 13 weeks (NTP, 1992).
- *Biological Plausibility and Coherence* – Exposure of rats to ethylbenzene alters hepatic expression of different cytochrome P450 isozymes, resulting in increased hydroxylation of testosterone (Yuan *et al.*, 1997a,b). It is biologically plausible that enzyme induction and increased testosterone hydroxylation could result in increased testosterone clearance and lower circulating testosterone levels, a mode of action that has been proposed for LCTs induced by oxazepam (Cook *et al.*, 1999). The progression of Leydig cells to cancer involves a continuum of four morphological stages, including (1) normal; (2) hyperplasia; (3) adenoma; and (4) carcinoma (Capen, 2001). If there is a true effect of ethylbenzene on the carcinogenic process in rat Leydig cells, it is a weak effect that involves a small exacerbation of a common lesion progression in male rats exposed to high concentrations of ethylbenzene.

Figure 8-5. Correlation Plot for LCT in Rats and Mice Exposed to Ethylbenzene



Although the precise mode of action for the possible association between ethylbenzene exposure and LCTs in male rats is not known, it does not appear to involve genotoxicity (see **Section 8.3.3.5**). Overall, there is insufficient information available to establish an MOA for LCTs in rats exposed to ethylbenzene with a high degree of confidence.

Are Key Events in the Animal MOA Plausible in Humans?

The key events for ethylbenzene producing LCTs in male rats are presented in **Table 8-8**. Several general qualitative differences between male rats and human indicate that the LCT tumors may not be relevant to humans, as has been discussed in several comprehensive reviews (Prentice and Meikle, 1995; Clegg *et al.*, 1997; Cook *et al.*, 1999; Klaunig *et al.*, 2003). These differences include the following:

- *Rats Lack Sex Hormone Binding Globulin (SHBG)* - In humans, the majority of serum testosterone is bound to SHBG. The absence of this protein in rats makes them more sensitive to perturbations in serum testosterone levels and subsequent effects.
- *Rat Leydig Cells Possess Additional Receptors* – Rat Leydig cells possess a receptor for gonadotropin releasing hormone (GnRH) (Cooke and Sullivan *et al.*, 1985), which is not present in humans (Clayton and Huhtaniemi, 1982).
- *Rat Leydig Cells Are Sensitive to Prolactin* – The LH receptors of rat Leydig cells are sensitive to prolactin (Zipf *et al.*, 1978), whereas receptors on human Leydig cells are not (Prentice *et al.*, 1992).

Based upon these fundamental differences between male rats and humans, the LCTs observed in rats are not expected to be relevant to human health risk assessment. This conclusion is supported by a number of negative epidemiology studies conducted for chemicals that have been shown to produce LCTs in male rats by various mechanisms (cadmium, ethanol, lactose, lead acetate, nicotine) (Cook *et al.*, 1999). Furthermore, hormonal imbalances and a number of clinical substances that cause increases in Leydig cell tumors in rats have not resulted in an increased incidence of Leydig cell neoplasia in man (Capen, 2001).

Table 8-8. Key Events in the MOA for Ethylbenzene-Induced Leydig Cell Tumors

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Exposure	Exposures to EB occur under controlled conditions in a laboratory setting	Exposures to EB can occur at the workplace, from consumer products, and from the environment	Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals	Rodent exposures are orders of magnitude higher than expected human exposure	None identified. Linearity is assumed
Absorption	EB is well absorbed following inhalation (Chin <i>et al.</i> , 1980) or ingestion (El Mastri <i>et al.</i> , 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsurata, 1982; Morgan <i>et al.</i> , 1991; Susten <i>et al.</i> , 1990).	EB is well absorbed following inhalation (Bardodej and Bardodejova, 1970; Engström and Bjurström, 1978; Åstrand <i>et al.</i> , 1978; Gromiec and Piotrowski 1984). and dermal (Dutkiewicz and Tyras, 1967) routes. EB is assumed to be well absorbed via ingestion.	None identified. The processes dictating absorption are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar.	None identified. Linearity is assumed
Distribution of ethylbenzene to liver	Absorbed EB is rapidly distributed to all tissues in animals exposed via inhalation (Chin <i>et al.</i> , 1980; Cappaert, 2000) and dermal routes (Susten <i>et al.</i> , 1990). Rapid distribution following ingestion is assumed.	Rapid distribution to all tissues is assumed.	None identified. The processes dictating distribution are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar	None identified. Linearity is assumed

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Enzyme induction	Induction of several P450 isozymes in rats (Yuan <i>et al.</i> , 1997a,b)	Enzyme induction is assumed	None identified	None identified.	Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation)
Perturbation of Circulating Testosterone	Increased hydroxylation of testosterone in rats (Yuan <i>et al.</i> , 1997a,b)	No evidence	Lack of sex hormone binding globulin in rats makes them more susceptible to serum testosterone perturbation than humans	None identified	Potential threshold events
Promotion/Progression of Leydig tumors		No evidence	Rat Leydig cells are prolactin sensitive, while human Leydig cells are insensitive to prolactin	Quantitative differences in Leydig cell receptors and serum LH half-life; Background rates for LCTs approaches ~100% in rats and ~0.0002% in humans	Potential threshold events

Taking into Account Kinetic and Dynamic Factors, Is the Animal MOA Plausible in Humans?

In addition to the qualitative differences listed above, several general quantitative differences between rat and human that may be pertinent to LCT formation are listed below.

- Human Leydig cells are 10- to 100-fold less sensitive than corresponding cells in the rat with respect to human chorionic gonadotropin-induced testosterone secretion and mitogenic response (Simpson *et al.*, 1987).
- Rat and human Leydig cells differ with respect to the number of leutenizing hormone, with rat cells possessing considerably more (~20,000/cell) than human cells (1500/cell) (Huhtaniemi, 1983).
- The half-life for circulating leutenizing hormone differs between rats and humans, with rats exhibiting a lower half-life (5-10 minutes) compared to humans (>100 minutes) (Caron *et al.*, 1994; De Groot *et al.*, 1995).
- Rats have a greater Leydig cell mass:blood volume ratio than do humans (Simpson *et al.*, 1987).
- The background rate for LCT is very high in male rats, approaching 100%, while very low in humans (~1 in 5 million or 0.0002%) (Capen, 2001). LCTs in humans are also different in cellular origin (Haseman and Arnold, 1990; Capen, 2001; Clegg *et al.*, 1997; Cook *et al.*, 1999).

Based upon these considerations, LCT is not considered an appropriate model for assessing the potential risk to human males of developing this rare testicular tumor (Capen, 2001).

Conclusion

A weak association between ethylbenzene exposure and LCT incidence has been reported in a single study in male rats (NTP, 1999). These lesions were entirely comprised of adenomas (no carcinomas observed), and may reflect a small exacerbation by ethylbenzene exposure of Leydig cell hyperplasia, which is common to male rats. Although a precise mode of action has not been established for ethylbenzene and LCT, a genotoxic mode of action can be safely ruled out (**Section 8.3.3.5**). A possible mode of action involving the induction of hepatic enzymes and perturbation of circulating testosterone levels is proposed here, but is not well supported by the literature. However, because of many well-documented qualitative and quantitative differences between rats and humans, the LCTs observed are not expected to be relevant to human health risk assessment. A recent comprehensive review published by international scientists concluded that LCT should not be regarded as risk for humans with substances fulfilling several defined criteria: *“For all of these [defined MOAs for LCT], clear quantitative differences exist between species, with rodents being more sensitive than humans (Clegg et al., 1997). Therefore, one can reasonably conclude that no-observable effect levels for the induction of LCTs in rodent bioassays provide an adequate margin of safety for protection of human health and that the data support a nonlinear mode of action (i.e.,*

threshold response). ... ***In conclusion, the data suggest that nongenotoxic compounds that induce LCTs in rats most likely have low relevance to humans under most exposure conditions because humans are quantitatively less sensitive than rats. Other investigators have come to a similar conclusion...***” Ethylbenzene fulfills all the above criteria: it is non-genotoxic; the tumor response was restricted to the highest dose tested (750 ppm, well above threshold for non-linear pharmacokinetic behavior and projected human exposures); and the MOE is many multiple orders of magnitude between the NOEL and well characterized human exposures (**Section 6**). Alternative to its quantitative use, the Leydig cell data can be used in a qualitative manner to provide some support the conclusion that ethylbenzene is a multisite carcinogen in rodents. However, this conclusion is already well supported by the results obtained for the kidney, liver, and lung tumors in rodents.

8.3.3.5 Consideration of Direct Genotoxicity as a Default MOA for All Tumor Types

Is the Weight of Evidence Sufficient to Establish the MOA in Animals?

Under a default MOA of direct genotoxicity for all rodent tumor types, the key events following exposure and systemic absorption of ethylbenzene are assumed as follows: (1) distribution of parent chemical to target tissues; (2) metabolism of ethylbenzene in tissues (lung, liver); (3) formation of adducts between reactive ethylbenzene metabolite(s) and DNA in target cells; (4) miscoding of DNA resulting in mutation; (5) alterations in cell growth control; and (6) progression to cancer. The weight of evidence for this MOA is considered below within the context of the modified Hill criteria below.

- *Strength of Association* – Overall, data regarding *in vitro* genotoxicity provide equivocal evidence for a direct genotoxic MOA, whereas, data are available from *in vivo* studies which do not support this MOA. With respect to mutagenicity, although some *in vitro* studies have reported positive results for ethylbenzene, the majority of the *in vitro* studies have yielded negative results (**Table 8-9**). More importantly, available *in vivo* studies for ethylbenzene indicate a lack of genotoxicity. Ethylbenzene has been shown to produce a positive and an ambiguous mutagenic effect of ethylbenzene in L5178Y tk^{+/-} mouse lymphoma cells (McGregor *et al.*, 1988; Wollny, 2000). However, negative results were reported for ethylbenzene in mouse lymphoma cells in a more recently conducted study (Seidel *et al.*, 2006). Ethylbenzene was shown to weakly induce sister chromatid exchanges in human lymphocytes (Norppa and Vainio, 1983), and induce micronuclei and cell transformation in Syrian hamster embryo cells (Gibson *et al.*, 1997; Kerckaert *et al.*, 1996). Positive results were generally observed at high, nonphysiologic concentrations in which significant cytotoxicity (reduced growth) was observed. However, ethylbenzene has proven non-mutagenic in *Salmonella typhimurium*, *Escherichia coli*, and *Saccharomyces cerevisiae* (Dean *et al.*, 1985; Florin *et al.*, 1980, Nestmann *et al.*, 1980; NTP, 1999; Nestmann and Lee, 1983). Similarly, ethylbenzene was negative in inducing sister chromatid exchanges or chromosomal aberrations in Chinese

hamster ovary cells (NTP, 1999). Ethylbenzene was also negative in producing chromosomal aberrations in rat liver epithelial cells (Dean *et al.*, 1985). Ethylbenzene has been reported to show no increase in micronucleated erythrocytes in mice (NTP, 1999; Mohtashamipur *et al.*, 1985). In an epidemiological study of ethylbenzene in a styrene plant, workers, who were also exposed to styrene, benzene, toluene and xylenes, were found to have no increase in sister chromatid exchanges, DNA adduct formation, total micronuclei (although kinetochore positive micronuclei were increased), or DNA single-strand breaks in their peripheral lymphocytes (Holz *et al.*, 1995).

- *Consistency of Association* – Data supporting a direct genotoxic MOA are inconsistent. Some *in vitro* studies have reported that ethylbenzene is weakly genotoxic (NTP, 1999; NTP, 1992a; IARC, 2000; McGregor *et al.*, 1988; Norppa and Vainio, 1983). However, ethylbenzene is primarily considered non-genotoxic/non-mutagenic. Various *in vivo* and *in vitro* studies have shown ethylbenzene lacks genotoxic potential (Dean *et al.*, 1985; Florin *et al.*, 1980; Nestmann *et al.*, 1980; NTP, 1999; NTP, 1992a; Nestmann and Lee, 1983; Holz *et al.*, 1995; Mohtashamipur *et al.*, 1985; Henderson *et al.*, 2007).
- *Specificity of Association* – No studies have been performed that focus specifically on the direct genotoxic effects of ethylbenzene in the target tissues identified above (kidney, liver, lung, testes). Given the generally negative results from available genotoxicity studies, a direct genotoxic MOA does not appear to be able to explain the species-, sex-, and tissue-specificity of the rodent tumors observed for ethylbenzene.

Table 8-9. Summary of Genotoxicity Studies.

<i>In Vivo</i> / <i>In Vitro</i>	Species (end point)	Exposure Conditions	Results		Reference(s)
			w/o Metabolic Activation	w/ Metabolic Activation	
<i>In Vivo</i>	Mouse (micronucleated erythrocytes)	13 week exposure up to 1000 ppm	-	NA	NTP, 1999
	Mouse (micronuclei in bone-marrow erythrocytes)	650 mg/kg-day IP (x2)	-	NA	Mohtashamipur <i>et al.</i> , 1985
	Human (sister chromatid exchange, DNA adducts, micronuclei, strand breaks in lymphocytes)	Occupational exposure	-	NA	Holz <i>et al.</i> , 1995
<i>In Vitro</i>	Mouse lymphoma (gene mutation)	Up to 120 µg/mL	-	-	Seidel <i>et al.</i> 2006
	Mouse lymphoma (gene mutation)	Up to 160 µg/mL	+	ND	NTP, 1999
	Human lymphocytes (sister chromatid exchange)	Up to 1061.6 mg/L	(+)	ND	Norppa and Vainio, 1983
	Syrian hamster embryo cells (micronuclei)	25 µg/ml	+	ND	Gibson <i>et al.</i> , 1997
	Syrian hamster embryo cells (cell transformation)	200 µg/ml	+	ND	Kerckaert <i>et al.</i> , 1997
	<i>Salmonella typhimurium</i> (gene mutation)	Up to 1,000 µg/plate	-	-	Zeiger <i>et al.</i> , 1992
	<i>S. typhimurium</i> (gene mutation)	Up to 2000 µg/plate	-	-	Dean <i>et al.</i> , 1985
	<i>S. typhimurium</i> (gene mutation)	Up to 3184 µg/plate	-	-	Florin <i>et al.</i> , 1980
	<i>S. typhimurium</i> , (gene mutation)	Up to 0.4 mg/plate	-	-	Nestman <i>et al.</i> , 1980
	<i>S. typhimurium</i> , (gene mutation)	Up to 1000 µg/plate	-	-	NTP, 1999; NTP, 1992a
	<i>Escherichia coli</i> , (gene mutation)	Up to 2000 µg/plate	-	-	Dean <i>et al.</i> , 1985
	<i>Saccharomyces cerevisiae</i> (gene mutation)	conc. not determined	-	-	Dean <i>et al.</i> , 1985
	<i>S. cerevisiae</i> (gene mutation)	conc. not determined	-	ND	Nestmann and Lee, 1983
	Chinese hamster ovary cells (sister chromatid exchange)	Up to 151-175 µg/ml	-	-	NTP, 1999; NTP, 1992a; IARC, 2000
	Chinese hamster ovary cells (chrom. aberrations)	Up to 150 µg/ml	-	-	NTP, 1999; NTP, 1992a
	Rat liver epithelial cells (chrom. aberrations)	conc. not determined	-	ND	Dean <i>et al.</i> , 1985

- Dose-Response Concordance* – Based upon the available data, the dose-response relationship for ethylbenzene genotoxicity does not provide concordance with the dose-response relationship for rodent tumorigenesis. *In vivo* studies for the genotoxicity of ethylbenzene are negative, despite the fact they include high exposures (up to 1000 ppm via inhalation, 650 mg/kg-day via ip injection). The few positive results for the genotoxicity of ethylbenzene in *in vitro* studies are associated with non-physiological concentration levels, and therefore their relevance to human health risk assessment is questionable. In human lymphocytes, sister chromatid exchanges were weakly increased at the highest (and cytotoxic) dose of 1,061.6 mg/L (Norppa and Vainio, 1983). In Chinese hamster ovary cells, mutations were negative at 75, 100, and 125 mg/L (NTP, 1999). *Salmonella typhimurium* exposed to 0, 10, 33, 100, 333, 666, and 1000 µg/plate ethylbenzene were negative for mutagenicity (NTP, 1992a; NTP, 1999). Ethylbenzene has demonstrated variable responses in the mouse lymphoma assay at the highest nonlethal dose (NTP, 1999; NTP, 1992a, IARC, 2000; McGregor *et al.*, 1988; Seidel *et al.*, 2006). According to McGregor *et al.* (1988), 80 mg/L was mutagenic and 100 mg/L was lethal to mouse lymphoma cells. However, a repeat study did not find a mutagenic response in mouse lymphoma cells with concentrations up to 120 mg/L (Seidel *et al.*, 2006). Mice exposed to 750 ppm showed no increase in micronucleated erythrocytes (NTP, 1999), and similarly, mice dosed with up to 645 mg/kg ethylbenzene were negative for micronuclei induction (Mohtashamipur *et al.*, 1985). Any positive responses observed at high concentrations need to be interpreted with caution since these high exposures to ethylbenzene are above the concentrations producing metabolic saturation.
- Temporal Relationship* – Thirteen weeks of ethylbenzene exposure resulted in no increase in micronucleated erythrocytes in mice (NTP, 1999) and 2 days intraperitoneal injection of ethylbenzene resulted in no increase in micronuclei (Mohtashamipur *et al.*, 1985). Occupational exposure (8-hour work shifts) reported no increase in sister chromatid exchanges, DNA adduct formation, micronuclei, or DNA single-strand breaks in their peripheral lymphocytes (Holz *et al.*, 1995). Forty-eight hour exposure to ethylbenzene resulted in a marginal increase in sister chromatid exchanges in human lymphocytes (Norppa and Vainio, 1983). Because genotoxicity was not observed in the *in vivo* studies, these data do not provide temporal concordance with the tumor data.
- Biological Plausibility and Coherence* – Considering that genotoxic effects were only seen at the highest non-lethal dose in a mouse lymphoma assay, but not in a more recent study using the same test system, and a weakly positive response was observed at only the highest concentration tested in human lymphocytes, indicate that direct genotoxicity is not a likely MOA for ethylbenzene-induced tumors in rats or mice. Any positive responses observed at high concentrations need to be interpreted with caution since these high exposures to ethylbenzene are above the concentrations producing metabolic saturation. Additionally, all *in vivo* studies were negative for genotoxicity. Ethylbenzene and its metabolites do not possess

any structural alerts for genotoxic potential (Henderson *et al.*, 2007). Therefore, a direct genotoxic MOA does not provide biological plausibility and coherence.

The key events for a genotoxic MOA for all tumors associated with ethylbenzene in rodents are presented in **Table 8-10**. In summary, all *in vivo* studies have been negative for genotoxicity and the *in vitro* studies have been predominantly negative for genotoxicity. Direct genotoxicity does not seem to be a relevant MOA for ethylbenzene - induced kidney, liver, or lung tumors in either rats or mice, and therefore is not to be relevant to human health risk assessment.

Table 8-10. Key Events in a Hypothetical Genotoxic MOA for All Ethylbenzene-Induced Tumors

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Exposure	Exposures to EB occur under controlled conditions in a laboratory setting	Exposures to EB can occur at the workplace, from consumer products, and from the environment	Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals	Rodent exposures are orders of magnitude higher than expected human exposure	None identified. Linearity is assumed
Absorption	EB is well absorbed following inhalation (Chin <i>et al.</i> , 1980) or ingestion (El Mastri <i>et al.</i> , 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsurata, 1982; Morgan <i>et al.</i> , 1991; Susten <i>et al.</i> , 1990).	EB is well absorbed following inhalation (Bardodej and Bardodejova, 1970; Engström and Bjurström, 1978; Åstrand <i>et al.</i> , 1978; Gromiec and Piotrowski 1984). and dermal (Dutkiewicz and Tyras, 1967) routes. EB is assumed to be well absorbed via ingestion.	None identified. The processes dictating absorption are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar.	None identified. Linearity is assumed
Distribution of ethylbenzene and metabolites to target tissues	Absorbed EB is rapidly distributed to all tissues in animals exposed via inhalation (Chin <i>et al.</i> , 1980; Cappaert, 2000) and dermal routes (Susten <i>et al.</i> , 1990). Rapid distribution following ingestion is assumed.	Rapid distribution to all tissues is assumed.	None identified. The processes dictating distribution are assumed to be qualitatively similar.	None identified. The rates and extents of absorption are assumed to be quantitatively similar	None identified. Linearity is assumed

Event	Evidence in Rodents	Evidence in Humans	Qualitative Differences Between Rodents and Humans	Quantitative Differences Between Rodents and Humans	Sources of Nonlinearity That May Impact High-to-Low Dose Extrapolation
Formation of DNA adducts by ethylbenzene or metabolites	No evidence	No DNA adducts were detected in peripheral lymphocytes of exposed workers (Holz <i>et al.</i> , 1995). A marginal increase in SCE was reported in human lymphocytes exposed <i>in vitro</i> (Norppa and Vainio, 1983)	None identified	None identified	None identified
DNA miscoding resulting in gene mutation	<i>In vivo</i> genotoxicity studies are negative (NTP, 1999; Mohtashamipur <i>et al.</i> , 1985). <i>In vitro</i> genotoxicity studies are largely negative with few positive results (see Table 8-8)	No DNA strand breaks or SCEs in exposed workers (Holz <i>et al.</i> , 1995)	None identified	None identified	None identified
Altered gene expression resulting in altered cell growth	No Evidence	No Evidence	None identified	None identified	None identified
Formation of preneoplastic lesions	No Evidence	No Evidence	None identified	None identified	None identified
Progression/Promotion of tumors	No Evidence	No Evidence	None identified	None identified	None identified

8.3.4 Dose-Response Assessment

A cancer dose-response assessment has not been prepared by U.S. EPA because at the time of the assessment (1991), the NTP cancer bioassay had not been conducted, and ethylbenzene was considered a Group D carcinogen (not classifiable as to human carcinogenicity). For this reason, a dose-response assessment was conducted for ethylbenzene for the purposes of deriving estimates of its cancer potency based upon the results obtained from rodent cancer bioassays. The dose-response assessment includes a number of decision points, include the selection the following: (1) Data Set; (2) Dose Measure, Response Measure; (3) Dose-Response Model; (4) Point of Departure; and (5) Low Dose Extrapolation Method. Each of these decisions is summarized below.

8.3.4.1 Data Set

Although adequate epidemiology data are not available for addressing the cancer potency of ethylbenzene, several tumor sites were identified in rodent cancer bioassays, including kidney, lung, and liver. Based upon a consideration of the MOAs summarized in Section 8.3.3, the kidney and liver tumors observed in rodents occur via processes that are not expected to occur in humans, and therefore are not considered relevant to human health risk assessment. For this reason, the lung tumors observed in male mice were identified as the basis for estimating the cancer potency of ethylbenzene. Dose-response data for lung tumors in mice are summarized in **Table 8-11**.

Table 8-11. Dose-Response Data for Lung Tumors Observed in Mice Exposed to Ethylbenzene

Concentration (ppm)	Lung Tumors (Male Mice)	
	Internal Dose (mg ethylbenzene metabolized/kg tissue/week)	Incidence
0	0	7/50
75	18,343	10/50
250	49,230	15/50
750	133,229	19/50

8.3.4.2 Dose Measure

Based upon the MOA described in Section 8.3.3 for ethylbenzene-induced lung tumors in mice, the concentration of catechol and quinone metabolites in tissue is expected to be proportionate to tissue tumor response. Because the PBPK model does not provide descriptions for individual metabolites, the total amount metabolized/kg tissue-week is used as an internal dose surrogate (Table 8-10). Details of the mouse PBPK model are found in Appendix P.

8.3.4.3 Response Measure

The dose-response data were assessed in terms of extra risk. Since mortality in exposed mice was similar to control animals, no adjustments for early mortality were required.

8.3.4.4 Dose-Response Model

A dose-response model was selected based upon several criteria: (1) visual inspection of the fit to the data; (2) value for AIC; (3) p-value obtained for goodness-of-fit; and (4) variation in the BMD estimate predicted by the model (indicated by the ratio of EC10/LEC10). Based upon these criteria (see **Table 8-12** below for the AIC and p-values), the multistage model was selected as an appropriate model for characterizing the dose-response relationship for the lung tumor data from male mice (**Figure 8-5**).

Table 8-12. Comparison of Models Fit to Ethylbenzene Lung Tumor Data

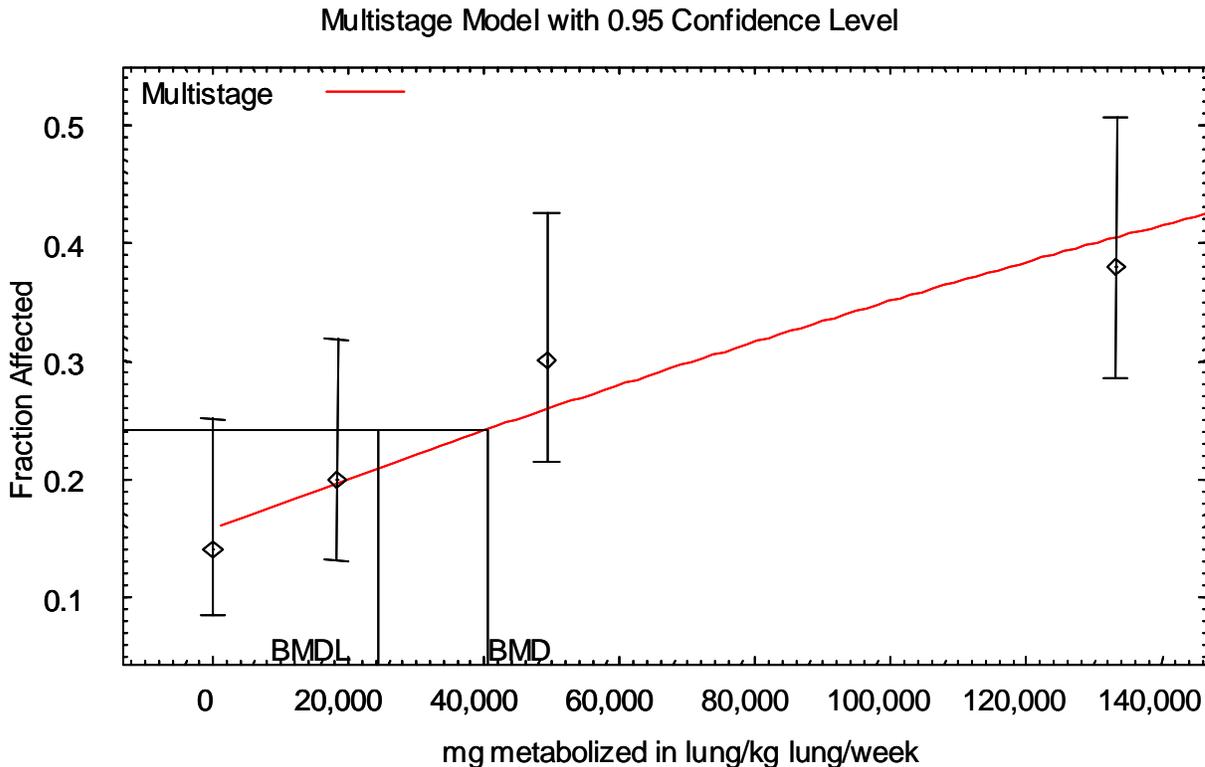
Model	Model Fit	
	AIC	P-Value
multistage	222.7	0.711
gamma	222.7	0.711
quantal linear	222.7	0.711
Weibull	222.7	0.711
probit	223.3	0.534
logistic	223.4	0.513
Log probit	224.2	0.718
Log logistic	224.2	0.683
quantal quadratic	224.6	0.275

8.3.4.5 Point of Departure

The concentration producing a 10% increase in tumor response (EC10) and its corresponding 95% lower and upper confidence limits (LEC10 and UEC10) was considered to be an appropriate point of departure for ethylbenzene. Although U.S. EPA's Benchmark Dose Software (BMDS version 1.3.2) does not calculate UEC10 values, these were estimated by assuming the distribution for the EC10 is symmetric (*i.e.*, $UEC10 = EC10 + (EC10 - LEC10)$). In instances where the EC10 distribution is skewed to the right, this approach will conservatively underestimate the true UEC10 value. The 10% benchmark response rate serves as the default point of departure as described by U.S. EPA guidelines (2005e).

For lung tumors, using the multistage model, the EC10, LEC10, and UEC10 values (expressed to 3 significant figures) were determined to be 40,500, 24,500 and 56,600 mg metabolized in lung/kg lung/week, respectively.

Figure 8-6. Fits of the Multistage Model to the Dose-Response Data for Lung Tumors in Male Mice



8.3.4.6 Extrapolation to Low Doses and Potentially Susceptible Subpopulations

Based upon a consideration of the MOA described in Section 8.3.3, the dose-response relationships for ethylbenzene -induced lung and liver tumors are expected to be nonlinear in nature, consistent with the existence of a threshold. Low-dose extrapolation was performed by the application of uncertainty factors as summarized below.

- **UFA** – A factor of 3 was considered appropriate to account for potential species differences in the toxicodynamics of ethylbenzene because a PBPK model was used to account for important species differences in the toxicokinetics of ethylbenzene.
- **UFH** – In the absence of specific information on human variation, a default factor of 10 was considered to be appropriate for ethylbenzene.
- **UFL** – A default value of 10 is recommended for **UFL** to account for the severity of the lesion on which the point of departure is based.
- **UFS** – Because the key study included a chronic exposure duration, **UFS** is not required (*i.e.*, **UFS**=1).
- **UFD** – Because the database for ethylbenzene is robust and includes chronic cancer bioassays in both rats and mice (NTP, 1999), **UFD** is not required (*i.e.*, **UFD**=1).

These UF values yield a composite UF of 300 (3x10x10x1x1).

8.3.4.7 Cancer Values

Cancer values for ethylbenzene based upon nonlinear extrapolation are provided below.

When the UF value of 300 is applied to the point of departure for lung tumors, a central tendency estimate of 135 mg metabolized/kg lung/week (EC10/300), a lower bound of 81.6 mg metabolized/kg lung/week (LEC10/300), and an upper bound of 188 mg metabolized/kg lung/week (UEC10/300) were calculated. The following cancer values were derived:

- *Using the analytical detection limit to derive the human lung metabolism rate (Appendix P)* - The PBPK model for ethylbenzene with a human lung metabolism estimate derived from the analytical detection limit for microsomal incubations (see Appendix P, Saghir and Rick, 2005) was used to predict the corresponding external concentrations and doses for continuous exposure associated with the internal dose levels derived above. The external concentrations are as follows: central tendency = 5.2 ppm; lower bound = 3.1 ppm; and upper bound = 7.6 ppm. The corresponding daily ingestion rates are 7.1, 4.3, and 9.9 mg/kg bwt/day, respectively.
- *Using a conservative estimate of human lung metabolism rate (Appendix P)* - The PBPK model for ethylbenzene with a human lung metabolism estimate derived from the rat lung Vmax determined using rat lung microsomes (see Appendix P, Saghir *et al.*, 2007) was used to predict the corresponding external concentrations and doses for continuous exposure associated with the internal dose levels derived above. The external concentrations are as follows: central tendency = 0.80 ppm; lower bound = 0.48 ppm; and upper bound = 1.1 ppm. The corresponding daily ingestion rates are 1.1, 0.71, and 1.6 mg/kg bwt/day, respectively.

8.3.5 Discussion

A cancer dose-response assessment was conducted for ethylbenzene. Although data from the NTP cancer bioassay for ethylbenzene in rats and mice indicate that ethylbenzene is carcinogenic at high doses, information regarding the MOA by which ethylbenzene produces tumors strongly impacts how these data should be applied to human health risk assessment. Information regarding the MOA for kidney tumors indicate a strong association with CPN, and therefore these tumors are not considered to be relevant to human health. Likewise, liver tumors in female mice appear to result from a phenobarbital-like MOA which is not relevant to humans. Information regarding the MOA for lung tumors support a role for oxidative metabolites (catechols, quinones), and because of species differences in the rates of ethylbenzene metabolism, the potency of ethylbenzene in humans is expected to be much lower than in laboratory rodents. Furthermore, the role of oxidative stress in the formation of these tumors supports a nonlinear dose-response relationship for tumor formation that is consistent with a threshold.

Several sources of conservatism are noted in this dose-response assessment. For example, use of the PBPK model here is considered conservative since the internal dose measure used in

the assessment only captures the initial oxidation of ethylbenzene, and subsequent ring-oxidation is also expected to show important species differences (humans < rodents).

Confidence in the cancer dose-response assessment for ethylbenzene is considered medium to high. Confidence in the key study (NTP, 1999) is considered high since it includes an adequate number of animals and test groups in both sexes of two species exposed for a lifetime. Confidence in the toxicity database is considered medium, primarily due to the lack of high quality epidemiology data. Confidence in the dosimetry is considered medium since the PBPK model for ethylbenzene addresses some of the important species differences in toxicokinetics (initial ring oxidation), but does not provide a description of the key oxidative metabolites (catechols, quinones). Confidence in the dose-response modeling is considered high since the multistage model provides excellent fits to the dose-response data for lung tumors.

8.4 Summary

An **RfC of 0.3 ppm** is proposed, based on ototoxicity observed in rats (Gagnaire *et al.*, 2007). This proposed RfC is slightly higher than the existing RfC (0.2 ppm), but can be assigned greater confidence (medium-to-high confidence) than the existing IRIS RfC (low confidence).

An **RfD of 0.5 mg/kg bwt/day** is proposed based on liver effects observed in the chronic mouse inhalation study (NTP, 1999). The hepatic effects seen in the chronic mouse inhalation study (NTP, 1999) and subchronic oral rat study (Mellert *et al.*, 2004, 2007) were similar. Use of the mouse inhalation study rather than the rat oral study obviates the need for an uncertainty factor for study duration (subchronic to chronic extrapolation) and increases confidence because the inhalation toxicity testing database is more extensive than the oral database. An identical RfD was derived using the rat ototoxicity study from which the RfC was derived and highly conservative uncertainty factors. Overall, the confidence in the proposed RfD is medium-to-high.

A **cancer reference value of 0.48 ppm** (lower bound; central tendency = 0.80 ppm; and upper bound = 1.1 ppm) was derived for ethylbenzene based upon an uncertainty factor of 300 applied to the points of departure for mouse lung tumors, respectively, and applying a conservative estimate of human lung metabolism. These concentrations correspond to **daily ingestion rates of 0.71 mg/kg bwt/day** (lower bound; central tendency = 1.1 mg/kg bwt/day; and upper bound = 1.6 mg/kg bwt/day).

9.0 RISK CHARACTERIZATION

9.1 Introduction

Chemical risk characterization is the integration of the chemical's exposure assessment and hazard assessment, wherein the toxicity reference values, based on the hazard assessment, provide a context for interpreting the exposure estimates. For this VCCEP assessment, quantitative risk characterizations were done for children and prospective parents exposed to ethylbenzene.

9.1.1 Summary of Exposure Assessment

As noted previously, acute exposure to ethylbenzene present as a component of mixed xylenes in consumer products was not formally considered in this assessment, but was estimated based on the analysis performed in the xylenes VCCEP submission (American Chemistry Council, 2005).

For ethylbenzene exposure to the general public through indoor air, outdoor air, and other source media, it was difficult to separate the contribution of the amount of ethylbenzene from the ethylbenzene/styrene chain of commerce, which is expected to be small, with that from the much larger refinery chain of commerce. In the refinery chain of commerce ethylbenzene is a byproduct of petroleum refining and is found in gasoline, other crude products, and mixed xylenes. Therefore, an additional objective of this assessment was to distinguish, on a semi-quantitative basis, that proportion of each exposure pathway that was directly attributable to the ethylbenzene/styrene chain of commerce. As discussed in Section 6, the proportion of ethylbenzene in ambient air that is attributable to the ethylbenzene/styrene chain of commerce cannot be precisely quantified, but a conservative estimate is thought to be 1%. Contribution of ethylbenzene attributable to the ethylbenzene/styrene chain of commerce to the population also exposed to ethylbenzene through smoking would be approximately 0.7%. The contribution of migration from food-contact material to the total dietary ethylbenzene concentration was conservatively estimated at 25%.

Estimated exposure concentrations in the identified media and population-specific exposure parameter values were used to estimate potential intake for children and prospective parents. Both the intake of ethylbenzene from exposure in the identified media from all sources of ethylbenzene and that portion of the total intake that could reasonably be attributed by ethylbenzene/styrene chain of commerce sources were considered. General intake equations for the inhalation pathway (due to exposure while at home, at school, outdoors, at work, and in a motor vehicle), dietary intake, ingestion of breast milk (for an infant), and mouthing of toys (for children) were used to estimate potential intake of ethylbenzene.

With children, with the exception of the age group <1 year, total intake by the inhalation pathway considered the amount of exposure at home, at school, outdoors, and riding in a motor vehicle. As expected, the contribution from the home represented 80% to greater

than 90% of the total ethylbenzene intake by the inhalation route for all age groups. The inhalation pathway (the sum of microenvironments) was the most significant contributor to total ethylbenzene intake in all microenvironments. The percent contribution was, as expected, greatest in the urban, smoking setting because the expected air concentrations, both outdoors and indoors, were the highest. The percent contribution was the lowest, as expected in the rural, non-smoking setting because the air concentrations were reduced but the contribution from the diet remained the same in each microenvironment.

Intake by way of the diet is a composite of the intake from all foods considered and was based on ethylbenzene concentrations in each food type and the age-specific ingestion rate for that food. The most significant contributor to diet for the bottle-fed infant was formula and whole milk, which were not assumed to be ingested by the breastfed infant. The contribution from breast milk for both the ethylbenzene worker's child and the nonworker's child was very small and the total estimated intake from diet was less than that estimated for a bottle-fed infant. As with the contribution from the diet, the air concentrations were lower in rural and non-smoking settings; therefore, the percent contribution from breast milk was higher but the absolute estimated intake did not differ. The daily exposure levels associated with toy mouthing were orders of magnitude lower than those associated with other exposure pathways. It was concluded that mouthing of styrenic toys is unlikely to be a significant source of children's exposure to ethylbenzene. For children, the total intake in all settings decreased with age, as expected because of the higher inhalation to body weight ratios for the younger age groups. The highest estimates of intake for the general public, as expected, were in the urban, smoking setting

Key findings of the exposure assessment for children were that inhalation of ethylbenzene exceeded ingestion, urban exposures exceed rural/suburban exposures, and exposures of children ages 0-2 years old exceed those of children from age 3 to 19. The highest "central tendency" estimated intake was for bottle-fed infants <1 year old in an urban, smoking setting (3.63×10^{-3} mg/kg bwt/day total; 2.64×10^{-3} mg/kg bwt/day from inhalation, 9.90×10^{-4} mg/kg bwt/day from diet). The highest "upper-bound" intake estimate was for an ethylbenzene production worker's breastfed child (8.10×10^{-3} mg/kg/day total; 5.87×10^{-3} mg/kg bwt/day from inhalation, 1.70×10^{-3} mg/kg bwt/day from breast feeding and 5.32×10^{-4} mg/kg bwt/day from diet).

Exposure pathways considered for prospective parents were inhalation of ethylbenzene - containing air in the workplace and other indoor, ambient, and motor vehicle environments and ingestion of food stuffs containing ethylbenzene. As with the higher end of the children age groups, inhalation was the dominant exposure pathway in the adult exposure scenarios, contributing at least 84% of the total intake. The influence of exposure setting on magnitude of adult exposure and relative contributions of the inhalation and ingestion pathways were similar to that discussed above for children. However, as expected, exposure for the production worker scenario was one to two orders of magnitude greater than those estimated for other adult populations due to the assumption of higher workplace exposure. The highest "central tendency" and "upper bound" exposures were for ethylbenzene production workers in an urban, smoking setting. These workers have negligible exposure to ethylbenzene from diet, as compared

to inhalation exposure. The central tendency estimate of inhalation exposure for this group was 0.0229 mg/kg bwt/day, and the upper –bound estimate was 0.223 mg/kg bwt/day.

9.1.2 Summary of Hazard Assessment

The toxicity of ethylbenzene has been extensively tested, and is discussed in greater detail in Section 7.

9.1.2.1 Noncancer Effects

Ethylbenzene has low acute toxicity. Consistent with the known effects of organic solvents which cause a general and non-specific depression of the nervous system, acute exposure to high concentrations of ethylbenzene can induce acute neurological effects. Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies. Ethylbenzene is a moderate subchronic repeated exposure toxicity hazard by inhalation or oral dosing with consistent effects to the rodent liver and kidney. The subchronic oral study also detected a minimal regenerative anemia and a reduction in prothrombin time, both of questionable significance. Specialized investigations of ethylbenzene effects on hearing indicate inhaled ethylbenzene can cause ototoxicity. Ototoxicity has been reported in a recent 13-week study in rats that found alterations in brainstem auditory evoked responses and outer hair cell morphology in rats at concentrations of 200 ppm and greater ethylbenzene. Life-time inhalation exposures ethylbenzene produced pathological lesions in the mouse liver, lung, thyroid, and pituitary gland. Rats that received lifetime exposures to ethylbenzene exhibited pathological changes to kidney, prostate gland, bone marrow, and liver. Ethylbenzene is not a teratogen or reproductive toxicant and is not (selectively) toxic to the developing nervous system. There is no evidence that ethylbenzene is harmful to the immune system.

An RfC of 0.3 ppm was proposed (Section 8), based on ototoxicity observed in rats (Gagnaire *et al.*, 2007). This proposed RfC is slightly higher than the existing RfC (0.2 ppm), but can be assigned greater confidence (medium-to-high confidence) than the existing IRIS RfC (low confidence).

An RfD of 0.5 mg/kg bwt/day was proposed (Section 8) based on liver effects observed in a chronic mouse inhalation study (NTP, 1999). The hepatic effects seen in the chronic mouse inhalation study (NTP, 1999) and subchronic oral rat study (Mellert *et al.*, 2004, 2007) were similar. Use of the mouse inhalation study rather than the rat oral study obviates the need for an uncertainty factor for study duration (subchronic to chronic extrapolation) and increases confidence because the inhalation toxicity testing database is more extensive than the oral database. Overall, the confidence in the proposed RfD is medium-to-high.

9.1.2.2 Carcinogenicity

Ethylbenzene is carcinogenic in animals following lifetime exposures to high vapor concentrations. Exacerbation by ethylbenzene of chronic progressive nephropathy, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the mode of action underlying the development of the rat renal cancer. Male rats that inhaled 750 ppm ethylbenzene also appeared to have an exacerbation in testicular tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence at 750 ppm ethylbenzene of liver and lung tumors in mice. The incidences of lung tumors in male mice and liver tumors in female mice were greater than those in concurrent control but were within the NTP historical control ranges. Increases in regenerative cell proliferation are postulated to play a key role in the mouse tumor findings.

A dose-response assessment was conducted for ethylbenzene with consideration of U.S. EPA's framework described in its *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005e), as described in Section 8.

A cancer reference value of 0.48 ppm (lower bound; central tendency = 0.80 ppm; and upper bound = 1.1 ppm) was derived for ethylbenzene based upon an uncertainty factor of 300 applied to the points of departure for mouse lung tumors, respectively, and applying a conservative estimate of human lung metabolism. These concentrations correspond to daily ingestion rates of 0.71 mg/kg bwt/day (lower bound; central tendency = 1.1 mg/kg bwt/day; and upper bound = 1.6 mg/kg bwt/day).

9.2 Risk Assessment Approaches

The risk characterization for ethylbenzene was performed using a hazard quotient (HQ) approach, as calculated using the following equation:

$$HQ = ADD / RfV$$

Where,

- HQ = Hazard quotient (unitless);
- ADD = Average daily dose, totaled for each route of exposure, as calculated in Section 6 (mg/kg bwt/day); and
- RfV = Reference value based upon noncancer or cancer endpoints derived in Sections 8.2 and 8.3 (mg/kg bwt/day).

The RfC (in ppm) was converted to units of mg/kg bwt/day by assuming using the adult inhalation rate of 16 m³/d and body weight of 71.8 kg (see Table 6-36).

A nonlinear cancer approach was considered appropriate for ethylbenzene based upon a consideration of the mode of action by which tumors are produced in rodents (Section 8.3). For this reason, the HQ approach was adopted for cancer endpoints as well as noncancer endpoints. HQs for the inhalation and ingestion routes of exposure were summed to calculate the hazard index (HI). An HI less than or equal to 1 is indicative that there is no elevated risk.

9.3 Ethylbenzene Risk Characterization

The toxicity reference values were used to assess the potential noncancer and cancer risks to children and adult populations exposed to ethylbenzene, as summarized in Tables 9-1 through 9-2. The risk characterizations for the most highly exposed groups are discussed in greater detail below.

In the xylenes VCCEP submission, modeled central tendency and upper-bound time-weighted average xylenes concentrations in indoor air for three time periods (one hour, eight hours, and 24 hours) were compared to the interim AEGL-1 for mixed xylenes (130 ppm). Resultant HIs ranged from 0.003 to 0.35, indicating no health concern based on xylene exposure (American Chemistry Council, 2005). As indicated in Section 1.2, mixed xylenes may contain 6 to 15% ethylbenzene, which was not accounted for in the xylenes submission. Conservatively assuming that the xylenes comprise 85% and ethylbenzene 15% of mixed xylenes in the modeled consumer products, the range of total mixed xylene concentrations in these scenarios was estimated as 0.2 to 54 ppm (Section 6.3.1.2). Dividing this range by the AEGL-1, the range of HIs would be 0.001 to 0.42. Thus, as concluded in the xylenes VCCEP submission, “the short-term exposure concentrations associated with the indoor use of xylenes as a degreaser or a component of spray paint in accordance with manufacturer instructions are unlikely to produce noticeable discomfort or irritation to the general public and susceptible individuals” (American Chemistry Council, 2005).

9.4 Chronic Risk Characterization for the Most Highly-Exposed Children

9.4.1 Noncancer

The central tendency estimates for bottle-fed urban infants (< 1 year old) in a smoking environment were HQs of 0.009 for inhalation and 0.002 for ingestion, for a total HI of 0.01. The upper bound estimates for a production worker’s breast-fed infant in an urban, smoking environment were HQs of 0.02 for inhalation and 0.004 for ingestion, for a total HI of 0.02. These HIs indicate that even the most highly-exposed children are not at risk for noncancer effects of ethylbenzene.

9.4.2 Cancer

The central tendency estimates for bottle-fed urban infants (< 1 year old) in a smoking environment were HQs of 0.004 for inhalation and 0.001 for ingestion, for a total HI of 0.005. The upper bound estimates for a production worker’s breast-fed infant in an urban, smoking environment were HQs of 0.008 for inhalation and 0.003 for ingestion, for a total HI of 0.01. These HIs indicate that even the most highly-exposed children are not at risk for lung cancer from ethylbenzene exposure.

9.5 Risk Characterization for the Most Highly-Exposed Prospective Parents

9.5.1 Noncancer

The central tendency estimates for production workers living in an urban, smoking environment were HQs of 0.08 for inhalation and 0.0001 for ingestion, for a total HI of 0.08. The upper bound estimates for these workers were HQs of 0.7 for inhalation and 0.0003 for ingestion, for a total HI of 0.7. These HIs indicate that even the most highly-exposed prospective parents are not at elevated risk for noncancer effects of ethylbenzene.

9.5.2 Cancer

The central tendency estimates for production workers living in an urban, smoking environment were HQs of 0.03 for inhalation and 0.0001 for ingestion, for a total HI of 0.03. The upper bound estimates for these workers were HQs of 0.3 for inhalation and 0.0004 for ingestion, for a total HI of 0.3. These HIs indicate that even the most highly-exposed prospective parents are not at elevated risk for lung cancer from ethylbenzene.

Table 9-1. Ethylbenzene Noncancer Hazard Characterization

Scenario	Assessment	Route	<1 year (Bottle-fed)	<1 year (Breastfed)	Worker's Child (Breastfed)	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years	At-Home Parent	Office Worker	Production Worker
Urban, Smoking Intake	Central Tendency	Inhalation	9E-03	9E-03	9E-03	9E-03	7E-03	6E-03	4E-03	3E-03	4E-03	3E-03	8E-02
		Oral	2E-03	6E-04	1E-03	1E-03	8E-04	4E-04	2E-04	2E-04	1E-04	1E-04	1E-04
		Total	1E-02	9E-03	1E-02	1E-02	8E-03	6E-03	4E-03	3E-03	4E-03	3E-03	8E-02
	Upper Bound	Inhalation	2E-02	2E-02	2E-02	2E-02	2E-02	1E-02	9E-03	7E-03	8E-03	7E-03	7E-01
		Oral	3E-03	1E-03	4E-03	3E-03	2E-03	9E-04	5E-04	5E-04	3E-04	3E-04	3E-04
		Total	2E-02	2E-02	2E-02	2E-02	2E-02	1E-02	1E-02	7E-03	8E-03	8E-03	7E-01
Urban, Non-Smoking	Central Tendency	Inhalation	6E-03	6E-03	6E-03	6E-03	5E-03	4E-03	3E-03	2E-03	2E-03	2E-03	8E-02
		Oral	2E-03	6E-04	1E-03	1E-03	8E-04	4E-04	2E-04	2E-04	1E-04	1E-04	1E-04
		Total	8E-03	7E-03	7E-03	7E-03	6E-03	4E-03	3E-03	2E-03	3E-03	2E-03	8E-02
	Upper Bound	Inhalation	1E-02	1E-02	1E-02	1E-02	1E-02	9E-03	6E-03	5E-03	6E-03	5E-03	7E-01
		Oral	3E-03	1E-03	4E-03	3E-03	2E-03	9E-04	5E-04	5E-04	3E-04	3E-04	3E-04
		Total	2E-02	1E-02	2E-02	2E-02	1E-02	9E-03	7E-03	5E-03	6E-03	5E-03	7E-01
Rural/Suburban, Smoking	Central Tendency	Inhalation	5E-03	5E-03	5E-03	5E-03	4E-03	3E-03	2E-03	2E-03	2E-03	2E-03	8E-02
		Oral	2E-03	6E-04	1E-03	1E-03	8E-04	4E-04	2E-04	2E-04	1E-04	1E-04	1E-04
		Total	7E-03	5E-03	6E-03	6E-03	5E-03	3E-03	2E-03	2E-03	2E-03	2E-03	8E-02
	Upper Bound	Inhalation	1E-02	1E-02	1E-02	1E-02	1E-02	8E-03	6E-03	4E-03	5E-03	5E-03	7E-01
		Oral	3E-03	1E-03	4E-03	3E-03	2E-03	9E-04	5E-04	5E-04	3E-04	3E-04	3E-04
		Total	2E-02	1E-02	2E-02	2E-02	1E-02	9E-03	6E-03	5E-03	5E-03	5E-03	7E-01
Rural/Suburban, Non-Smoking	Central Tendency	Inhalation	3E-03	3E-03	3E-03	3E-03	3E-03	2E-03	2E-03	1E-03	1E-03	1E-03	7E-02
		Oral	2E-03	6E-04	1E-03	1E-03	8E-04	4E-04	2E-04	2E-04	1E-04	1E-04	1E-04
		Total	5E-03	4E-03	4E-03	5E-03	3E-03	3E-03	2E-03	1E-03	1E-03	1E-03	7E-02
	Upper Bound	Inhalation	8E-03	8E-03	8E-03	9E-03	7E-03	6E-03	4E-03	3E-03	3E-03	3E-03	7E-01
		Oral	3E-03	1E-03	4E-03	3E-03	2E-03	9E-04	5E-04	5E-04	3E-04	3E-04	3E-04
		Total	1E-02	9E-03	1E-02	1E-02	8E-03	7E-03	5E-03	4E-03	4E-03	4E-03	7E-01

Table 9-2. Ethylbenzene Cancer Hazard Characterization

Scenario	Assessment	Route	<1 year (Bottle-fed)	<1 year (Breastfed)	Worker's Child (Breastfed)	1-2 years	3-5 years	6-8 years	9-14 years	15-19 years	At-Home Parent	Office Worker	Production Worker
Urban, Smoking Intake	Central Tendency	Inhalation	4E-03	4E-03	4E-03	4E-03	3E-03	2E-03	2E-03	1E-03	2E-03	1E-03	3E-02
		Oral	1E-03	5E-04	7E-04	9E-04	5E-04	3E-04	1E-04	1E-04	1E-04	1E-04	1E-04
		Total	5E-03	4E-03	4E-03	5E-03	3E-03	3E-03	2E-03	1E-03	2E-03	1E-03	3E-02
	Upper Bound	Inhalation	8E-03	8E-03	8E-03	8E-03	7E-03	5E-03	4E-03	3E-03	4E-03	3E-03	3E-01
		Oral	2E-03	8E-04	3E-03	2E-03	1E-03	7E-04	3E-04	3E-04	2E-04	2E-04	2E-04
		Total	1E-02	9E-03	1E-02	1E-02	8E-03	6E-03	4E-03	3E-03	4E-03	3E-03	3E-01
Urban, Non-Smoking	Central Tendency	Inhalation	3E-03	3E-03	3E-03	3E-03	2E-03	2E-03	1E-03	9E-04	1E-03	9E-04	3E-02
		Oral	1E-03	5E-04	7E-04	9E-04	5E-04	3E-04	1E-04	1E-04	1E-04	1E-04	1E-04
		Total	4E-03	3E-03	3E-03	3E-03	3E-03	2E-03	1E-03	1E-03	1E-03	1E-03	3E-02
	Upper Bound	Inhalation	6E-03	6E-03	6E-03	6E-03	5E-03	4E-03	3E-03	2E-03	2E-03	2E-03	3E-01
		Oral	2E-03	8E-04	3E-03	2E-03	1E-03	7E-04	3E-04	3E-04	2E-04	2E-04	2E-04
		Total	8E-03	6E-03	9E-03	7E-03	6E-03	4E-03	3E-03	2E-03	3E-03	2E-03	3E-01
Rural/Suburban, Smoking	Central Tendency	Inhalation	2E-03	2E-03	2E-03	2E-03	2E-03	1E-03	1E-03	7E-04	9E-04	8E-04	3E-02
		Oral	1E-03	5E-04	7E-04	9E-04	5E-04	3E-04	1E-04	1E-04	1E-04	1E-04	1E-04
		Total	3E-03	2E-03	3E-03	3E-03	2E-03	2E-03	1E-03	9E-04	1E-03	9E-04	3E-02
	Upper Bound	Inhalation	5E-03	5E-03	5E-03	5E-03	4E-03	3E-03	3E-03	2E-03	2E-03	2E-03	3E-01
		Oral	2E-03	8E-04	3E-03	2E-03	1E-03	7E-04	3E-04	3E-04	2E-04	2E-04	2E-04
		Total	7E-03	6E-03	9E-03	7E-03	5E-03	4E-03	3E-03	2E-03	2E-03	2E-03	3E-01
Rural/Suburban, Non-Smoking	Central Tendency	Inhalation	1E-03	1E-03	1E-03	1E-03	1E-03	9E-04	7E-04	5E-04	6E-04	5E-04	3E-02
		Oral	1E-03	5E-04	7E-04	9E-04	5E-04	3E-04	1E-04	1E-04	1E-04	1E-04	1E-04
		Total	3E-03	2E-03	2E-03	2E-03	2E-03	1E-03	8E-04	7E-04	7E-04	6E-04	3E-02
	Upper Bound	Inhalation	4E-03	4E-03	4E-03	4E-03	3E-03	2E-03	2E-03	1E-03	1E-03	1E-03	3E-01
		Oral	2E-03	8E-04	3E-03	2E-03	1E-03	7E-04	3E-04	3E-04	2E-04	2E-04	2E-04
		Total	6E-03	4E-03	7E-03	5E-03	4E-03	3E-03	2E-03	2E-03	2E-03	2E-03	3E-01

9.6 Sources of Uncertainty in Risk Characterization

Sources of uncertainty in the exposure assessment and toxicity assessment used in the risk characterization are discussed below.

9.6.1 Uncertainty in Exposure Assessment

The process of exposure assessment inherently involves uncertainties in the data selected, the populations and pathways described, and the values used to quantify exposure. Uncertainties in this assessment were broadly divided into two categories: (1) those associated with determining the concentration of ethylbenzene in relevant environmental media or other media (e.g., food or toys); and, (2) those associated with the values for those parameters used to describe population contact rates (e.g., frequency and amount) and characteristics (e.g., breathing rate and body weight). The approach taken was to make generally conservative assumptions, such that potential exposures may be overestimated, but are not likely to be underestimated.

Uncertainty in Inhalation Exposure Estimates

This assessment demonstrated that the dominant route of exposure to ethylbenzene is inhalation. Because both children and adults spend most of their time in buildings, concentrations in indoor air were the primary determinants of exposure. Levels of ethylbenzene in indoor air are highly dependent upon and dominated by outdoor sources, especially in urban environments, but indoor sources such as ETS likely contribute as well. Two major sources of uncertainties in the inhalation exposure assessment are therefore (1) the estimates of urban and rural outdoor air concentrations, and (2) the I/O ratios used to estimate exposure concentrations for each indoor microenvironment (home, office, school, motor vehicle).

Concentrations in outdoor air were obtained from the EPA's Air Quality System (AQS) database, which contains yearly summaries of data collected from all 50 states plus the District of Columbia, Puerto Rico and the Virgin Islands. Approximately 1,000 samples collected during the years 2000 to 2004 (excluding the year 2002 due to quality issues) were used to derive estimates of the central tendency and upper bound air concentrations. In the database, air monitors were classified as urban, suburban, or rural, but not identified by geographical area. Thus, specific areas of the country may have air concentrations that are higher or lower than the estimates. For example, industrial facilities that could be sources of ethylbenzene to the environment, such as refineries, are typically located in less densely populated areas. Monitors located in close proximity to such facilities would likely register higher air concentrations than other typical rural areas. Depending on the number and concentrations of these samples, their inclusion in the overall estimate could result in overestimation of concentrations in rural areas in general, while concentrations near emitting facilities would be underestimated. Underestimation of ambient ethylbenzene concentrations in this assessment is considered unlikely, however, as concentrations in urban and suburban areas decreased approximately 15% and 40%, respectively, from 2000 to 2003 (see Figure 6-2), while levels in rural areas remained fairly consistent at around $0.2 \mu\text{g}/\text{m}^3$. Thus, using data

from 2000 to 2004 may have overestimated the current levels for urban settings. Because suburban and rural concentrations were combined, current levels in rural areas were probably overestimated.

A fundamental difficulty encountered in exposure assessments based on ambient monitoring data is the fact that personal exposure levels (i.e., exposure levels determined by measurements taken in immediate proximity to the receptor) are almost always higher than measurements taken in living spaces, suggesting that individuals must spend some of their time in unmonitored zones of especially high exposure. In some cases, such zones consist of the person's own body. Therefore, an effort was made in this assessment to capture relatively higher exposures to ethylbenzene by including riding in automobiles and exposure to ETS. Exposure concentrations in indoor microenvironments were assumed to be multiples of outdoor concentrations based on published I/O ratios. As a result, uncertainties in estimates of outdoor air concentrations directly contribute to uncertainties in estimates of indoor air concentrations. Further, the accuracy of I/O ratios is limited by the small number of relevant studies. The estimated I/O for homes ranged from 1.1 to 5.6, with a mean of 3.1, based on several small studies in urban areas. All participants were non-smokers, but other contributors to indoor air concentrations, such as heating and cooking methods and the presence or absence of attached garages, were not considered in every study. Similarly, the data available to estimate indoor air concentrations in schools and offices were limited. These I/O ratios were generally lower than those for residences, as expected due to the lack of known contributors such as attached garages. The I/O ratio for offices in an urban area was derived from a single study in San Francisco, while data from across the U.S. were used as the I/O ratio for rural settings. The representativeness of these I/Os for individual indoor microenvironments is therefore uncertain, but application of the average of the range of I/O values to both central tendency and upper-bound estimates of outdoor air concentrations is considered more likely to result in over- than underestimation of indoor air concentrations. Although the available data do not unambiguously establish ETS as an important source for indoor air, it appears capable of increasing ethylbenzene concentrations in both air and blood. Therefore, contributions from ETS were also estimated for all indoor environments except schools by applying a multiplier to outdoor air concentrations. Because many industrial workplaces and offices are now smoke-free, this assumption would overestimate air concentrations in those microenvironments.

Not explicitly considered were exposures to ethylbenzene via use of mixed xylenes-containing household products such as paints, varnishes, and cleaning products or emissions from styrenic materials. However, exposures to mixed xylenes-containing products were quantitatively evaluated in the xylenes VCCEP submission (American Chemistry Council, 2005). As discussed in Section 9.3, hazard indices remain below 1 if inclusion of ethylbenzene in these calculations is conservatively assumed to increase mixed xylenes exposure concentrations by 15%. Further, it is reasonable to assume that typical indoor exposures to ethylbenzene represented in published I/O ratios for typical homes incorporated all sources.

Uncertainty in Dietary Intake Estimates

Ethylbenzene was infrequently detected in raw and processed foods in the U.S., and when detected, levels were in the parts-per-trillion range. It is generally not possible to distinguish the unique contributions from the major identified sources, i.e., diffusion from the atmosphere styrenic packaging. Therefore, it was assumed that the available measured food levels reflected ethylbenzene inputs from all sources.

Several assumptions made in this assessment are likely to overestimate ethylbenzene intake from food. Ethylbenzene levels in various food products from the FDA's TDS were assumed to be representative of all foods in that category, such as eggs, when in fact ethylbenzene was measured in only 5% of the eggs sampled. It was also assumed that each individual ingested all of the foods in the dietary list daily. While one or more food items from each of these categories may be eaten by an individual in a given day, it is highly improbable that every food listed in every category would be consumed daily by all individuals. In order to evaluate the contribution from food-contact materials, the total dietary intake was compared to the intake estimated using the food concentration term derived from Lickly et al.'s (1995) kinetic migration model (0.45 µg/kg). The model's assumption that the potential migration is 100% efficient is likely to result in overestimation of exposure to volatile chemicals such as ethylbenzene. Because the initial data for ethylbenzene levels in various styrenic products is about a decade old, it is uncertain how representative it is of materials currently on the market. Finally, it was assumed that none of the ethylbenzene present in foods was lost during storage or even cooking, and that all of the ethylbenzene ingested was absorbed into the systemic circulation.

Uncertainty in Other Media Estimates

It is recognized that petroleum leaks and spills and other releases can result in localized contamination of soil, sediment, surface water, and groundwater. However, such discrete exposure conditions are not reflective of those experienced on a long-term basis by the general public. Thus, potential exposures via contact with surface water, and groundwater were not quantified in this assessment because substantial national databases for these media indicate that the pervading condition is very low concentrations and detection frequencies. Ingestion of ethylbenzene by way of mouthing of toys and other objects in young children was based on a number of conservative assumptions and is unlikely to underestimate intake by this pathway. This pathway contributed very little to estimates of total intake and any under- or overestimate is not expected to have a significant impact on total estimates of intake.

Uncertainties in Parameter Values

Because inhalation and dietary intake were the major routes of exposure for all but those pathways unique to children, the parameter values that characterize these populations were limited to age-specific inhalation and ingestion rates and age-specific body weights. The values selected were those typically used in risk assessments derived from applicable EPA

and FDA guidance. While it could be argued that these tend to be on the conservative side, these values were considered appropriate for this screening assessment. Two assumptions with regard to parameter values had a significant impact on estimates of exposure. First, air concentrations were converted to estimates of intake in mg/kg-day without consideration of the amount of ethylbenzene that is absorbed in the lung and retained in the body. Because the inhalation route is the overwhelming contributor to total intake, this assumption results in significant overestimates of the contribution by this route. Implicit in the manner in which this was calculated is that all of the inhaled ethylbenzene is absorbed through the lung and that the kinetic fate of ethylbenzene is the same by the oral and inhalation routes. The former assumption is highly conservative and clearly results in an over-estimate of exposure. The latter assumption does not consider the potential impact of first pass metabolism, which could also result in an overestimate of dose (as opposed to intake) if the parent compound is responsible for potential toxicity. While it could be assumed that the estimated intake would be reduced by the fraction absorbed, i.e., if only 25% were absorbed, then total intake estimates for the inhalation route would be one-fourth.

Other Uncertainties

There appear to be no significant differences between children and adults in terms of exposure potential; the only exposure pathways unique to children are breastfeeding and mouthing of polystyrene toys. Estimated exposures via these pathways were small relative to those associated with inhalation and the diet. As discussed in Section 6.7.1.2, ethylbenzene/styrene chain of commerce was assumed to contribute one one-hundredth of the general public's total inhalation exposure to ethylbenzene through the inhalation pathway. As this fraction was determined using measured ambient air data and, as indicated above, that the use of household products or storage of gasoline may contribute to indoor air concentrations, the use of 1% of the indoor air concentration may result in an overestimate of intake.

Estimates of intake for adults were less than those estimated for the most highly exposed child's age group but were similar to those estimated for children aged 9 years and up for the at home parent and office worker and for children aged 3 years and up for the production worker. These results are different than those that could be expected from the ethylbenzene blood concentration data presented in Sexton *et al.* (2005), which reported children's ethylbenzene blood concentrations from their study were at least a factor of 1.5 lower than blood concentrations of adults participating in the NHANES study. The difference could be the result of the conservative assumptions, particularly for children, used in the estimation of ethylbenzene intake, or due to kinetic differences in adults and children that would not be reflected in estimates of intake based on external measures of exposure, or the overall decline in blood levels in the general population with the decreasing ambient concentrations from the years the NHANES population was sampled and the time that these children were evaluated.

9.6.2 Uncertainty in Toxicity Assessment

Study/Endpoint Selection

As described in Section 7, the toxic effects of ethylbenzene have been well studied in animals. A variety of endpoints were considered for the RfC (ototoxicity, liver effects), RfD (liver effects, hematological effects, ototoxicity), and the cancer value. With the exception of kidney, testicular, and liver tumors, all other endpoints observed for ethylbenzene were conservatively assumed to be potentially relevant to human health. For each toxicity value, care the study/endpoint resulting in the smallest (more health protective) reference value was generally taken as the key study. [The exception was the RfD, where the toxicity value was derived using a chronic inhalation study rather than a subchronic oral study.] Therefore, although uncertainty remains regarding which study/endpoint is the most appropriate for human health risk assessment, consideration of alternative studies/endpoints generally results in higher (less health protective) toxicity values.

Mode of Action

The MOAs by which ethylbenzene produces adverse effects in animals were assessed in Sections 8.2 and 8.3. Because information on MOA is used to guide key decisions in the dose-response assessments (relevance to human health, dose measure, low-dose extrapolation method), uncertainty in the MOA can have a large impact on the results.

Regarding the noncancer endpoints, several endpoints were considered. The proposed RfC was based on ototoxicity and the RfD was based on liver toxicity. Any uncertainty in the MOA for the key study is mitigated by the fact that other potential RfCs, derived for other endpoints with a different dose metrics, yielded equal or larger potential RfCs.

PBPK Modeling

Because PBPK modeling accounts for some species differences, incorporation of PBPK modeling is expected to reduce the uncertainty associated with interspecies extrapolation. Regarding the dose-response assessment for liver tumors, use of the PBPK model here is considered conservative since the internal dose measure used in the assessment only captures the initial oxidation of ethylbenzene, and subsequent ring-oxidation is also expected to show important species differences (humans < rodents).

Uncertainty Factor Selection

For all toxicity values derived, uncertainty factors of up to ten each were applied to account for interspecies variation (UFA), intraspecies variation (UFH), subchronic-to-chronic extrapolation (UFS), LOAEL-to-NOAEL (UFL), and/or database insufficiency (UFD). By their very nature, the application of these uncertainty factors is health protective since they reflect uncertainty in only one direction, where in reality some uncertainties are bidirectional. For example, a value of three was applied for UFA based upon consideration that humans may be three times more sensitive to the effects of ethylbenzene than is the test species due to toxicodynamic differences. However, it is equally plausible that humans are three times less sensitive than the test species. Similarly, a value of ten was applied based upon a consideration that an individual may be ten times more sensitive to the effects of

ethylbenzene than the average individual. However, it is equally plausible that an individual is ten times less sensitive than the average individual.

10.0 VCCEP DATA NEEDS ASSESSMENT

10.1 Hazard

The toxicological effects of ethylbenzene have been thoroughly studied. Ethylbenzene has been evaluated by all the toxicity tests listed under Tier 1, Tier 2, and Tier 3 of the Pilot Announcement and overall this information is of suitable quality to support human health hazard and risk assessments for children and prospective parents (Table 7-1). Specialized investigations of ethylbenzene effects on hearing do indicate ethylbenzene can cause ototoxicity. Additional investigation to further characterize the dose-response relationship between ethylbenzene and ototoxicity and the biological significance of certain measures of auditory response may be helpful to clarify hearing effects; however, the current VCCEP assessment has used a conservative interpretation of the biological significance of ototoxicity findings and hence no impact on the overall VCCEP assessment is anticipated from further ethylbenzene ototoxicity investigations.

10.2 Exposure

The exposure assessment herein is adequate to describe current exposures for children and prospective parents. As ethylbenzene air concentrations in urban and suburban settings show steady declines (while rural concentrations remain steady), future exposure data are likely to be lower than the data used in this assessment, thus providing a conservative assessment of human health risk.

10.3 Risk

The risk assessment was conducted using EPA guidance. The calculated HIs indicate that even the most highly-exposed children and prospective parents are not at risk for noncancer or cancer effects of ethylbenzene. Therefore, further evaluations of risks of ethylbenzene under VCCEP are unnecessary.

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