2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ETHYLBENZENE IN THE UNITED STATES

Ethylbenzene is widely distributed in the environment. It is primarily used for the production of styrene, which is the monomeric unit for polystyrene materials. Ethylbenzene is also used as a solvent and in the manufacture of several organic compounds other than styrene; however, these uses are very minor in comparison to the amounts used for styrene production. The production volume of ethylbenzene is typically among the highest of all chemicals manufactured in the United States. In 2005, nearly 12 billion pounds of ethylbenzene were produced domestically, with historical levels ranging anywhere from approximately 7 to 13 billion pounds annually. Routine human activities, such as driving automobiles, boats, or aircraft, and using gasoline powered tools and equipment as well as paints, varnishes, and solvents release ethylbenzene to the environment. Environmental and background levels of ethylbenzene are generally small and therefore, have minimal impact on public health. Trace levels of ethylbenzene are found in internal combustion engine exhaust, food, soil, water, and tobacco smoke, but usually at levels well below those that have been shown to exhibit toxic effects in laboratory animals or human exposure studies.

Ethylbenzene is not considered highly persistent in the environment. It partitions primarily to air and removal via photochemically generated hydroxyl radicals is an important degradation mechanism. The half-life for this reaction in the atmosphere is approximately 1–2 days. Biodegradation under aerobic conditions and indirect photolysis are important degradation mechanisms for ethylbenzene in soil and water. Based on a vapor pressure of 9.53 mm Hg and Henry’s law constant of 7.9x10^{-3} atm-m^3/mol, volatilization from water and soil surfaces is expected to be an important environmental fate process for ethylbenzene. If released to soil, ethylbenzene is expected to possess moderate mobility based on a soil adsorption coefficient (K_d) value of 240.

Ethylbenzene is ubiquitous in ambient air, primarily as a result of automobile emissions. The median level of ethylbenzene in city and suburban air was reported as 2.7 μg/m^3 (0.62 ppb). In contrast, the median level of ethylbenzene measured in rural locations was 0.056 μg/m^3 (0.013 ppb). Ethylbenzene levels in indoor air tend to be higher than corresponding levels monitored in outdoor air, as a result of contributions from environmental tobacco smoke (ETS) and various consumer products, in addition to the permeation indoors of ethylbenzene from attached garages or outside air. One study analyzed the components of ETS for the 50 top-selling U.S. cigarette brand styles in 1991 and for the University of
Kentucky Research cigarette, K1R4F. The ethylbenzene concentrations measured were 8.68 μg/m³ for full-flavor cigarettes, 8.24 μg/m³ for full-flavor, low-tar cigarettes, and 8.72 μg/m³ for ultra-low-tar cigarettes. The mean ethylbenzene concentration for all cigarettes was 8.50 μg/m³. One study reported a maximum outdoor air concentration of 7.4 μg/m³ (1.7 ppb) for ethylbenzene at four residential locations, while indoor air concentrations at these same homes ranged from 5 to 110 μg/m³ (1–25.3 ppb). Ethylbenzene is also released to the air during processing of crude oil.

Ethylbenzene is detected infrequently in surface water. Data from the EPA STOrage and REtrieval Database (STORET), indicated that ethylbenzene was detected in <3% of the surface water samples analyzed in the United States from January 2005 to March 2007, with a maximum concentration of 2 ppb. Ethylbenzene can also be present in groundwater, particularly near current or former landfills, hazardous sites, or gas stations. Oil- and gasoline-contaminated sites have also been found to have relatively high ethylbenzene concentrations in soil.

Ethylbenzene was identified in 82 different food items at a maximum concentration of 0.129 ppm in data obtained from the FDA Total Diet Study Market Basket Surveys collected between September 1991 and October 2003. Trace concentrations of ethylbenzene have been reported in split peas (0.013 mg/kg [ppm]), lentils (0.005 mg/kg [ppm]), and beans (mean concentration 0.005 mg/kg [ppm]; maximum concentration 0.011 mg/kg [ppm]).

The general population is primarily exposed to ethylbenzene from the inhalation of ambient air. This is due to the direct release of ethylbenzene into the air by the burning of fossil fuels or industrial processes, and partitioning into the air from other media (e.g., soil, surface water). This partitioning of ethylbenzene into the air or water would play a role in exposure to populations living near hazardous waste sites. In addition to inhalation exposure, ingestion of ethylbenzene may also occur because trace amounts have been found in water supplies and various food items.

### 2.2 SUMMARY OF HEALTH EFFECTS

In humans, eye irritation was observed after exposure to 10,000 ppm ethylbenzene for a few seconds. Volunteers reported irritation and chest constriction after acute-duration exposures to 2,000 ppm ethylbenzene. These symptoms worsened as the concentration was increased to 5,000 ppm. Human exposures in the range of 2,000–5,000 ppm ethylbenzene were associated with dizziness and vertigo. Complete recovery occurs if exposure is not prolonged. Momentary ocular irritation, a burning sensation,
and profuse lacrimation were observed in humans exposed to 1,000 ppm ethylbenzene. Workers exposed occupationally to solvent mixtures that included ethylbenzene showed an increased incidence of hearing loss compared to unexposed individuals. Respiratory effects were not observed in two patients exposed to 55.3 ppm ethylbenzene for 15 minutes. An increase in the mean number of lymphocytes and a decrease in hemoglobin levels were observed during a 1-year period in workers exposed chronically to solvents including ethylbenzene. However, no adverse hematological effects were observed in workers exposed to ethylbenzene for 20 years. Although no information on ethylbenzene concentrations was reported, an estimated concentration of 6.4 mg/m³ was derived from a mean post-shift in urinary mandelic acid concentration in workers, based on the relationship between ethylbenzene concentrations in air and urinary mandelic acid concentration in a chamber-exposed group. No liver lesions or differences in liver function tests between exposed and nonexposed workers were observed and no cases of malignancy in workers were reported. However, given the low exposure concentration, this study had limited the power to detect any effect. No other studies in humans exposed to ethylbenzene were located. Given that little data in humans are available, it is assumed that adverse effects observed in animals are relevant to humans.

Acute-duration and intermediate-duration studies in animals suggest that the auditory system is a sensitive target of ethylbenzene toxicity. Significant losses of outer hair cells (OHCs) in the organ of Corti have been observed in rats after acute-duration exposure ≥400 ppm and intermediate-duration inhalation exposure to ≥200 ppm ethylbenzene. These OHC losses have been observed up to 11 weeks after termination of the exposure, suggesting that these effects may be irreversible. Significant deterioration of auditory thresholds is also observed in animals affected with OHC losses. Auditory deficits have also been observed in animals after an intermediate-duration oral exposure to ethylbenzene. An almost complete loss of the three rows of outer hair cells in the organ of Corti was observed in rats 10 days after the last dose (900 mg/kg/day) in an acute-duration study. Effects on the central nervous system, such as moderate motor activation, narcotic effects, changes in posture and arousal, and salivation and prostration have been observed in animals after acute- and intermediate-duration exposure to ≥400 ppm ethylbenzene.

Results of 4- and 13-week studies indicate that intermediate-duration oral exposure to ethylbenzene produces effects to the liver. Effects indicative of liver toxicity observed included increased activity of serum liver enzymes (alanine aminotransferase and \(\gamma\)-glutamyl transferase) in males (≥250 mg/kg/day) and females (750 mg/mg/day), increased absolute and relative liver weights (≥250 mg/kg/day in males and females), and a dose-related increase in the incidence of centrilobular hepatocyte hypertrophy.
2. RELEVANCE TO PUBLIC HEALTH

(≥250 mg/kg/day in males and females). Increased bilirubin (≤250 mg/kg/day in males and 750 mg/kg/day in females), total protein (750 mg/kg/day in females), albumin (750 mg/kg/day in males and females), globulins (750 mg/kg/day in females), and cholesterol (≤250 mg/kg/day in males and females), and decreased prothrombin time (750 mg/kg/day in males and ≥250 mg/kg/day in females) were considered by study investigators as adaptive effects in the liver. In males in the 75 mg/kg/day group, relative liver weight was significantly increased by 4% compared to controls; however, no histopathological changes, or increases in absolute liver or serum liver enzyme activities were observed at this dosage. Given that ethylbenzene is a microsomal enzyme inducer, and the absence of histopathology and other evidence of liver injury at the 75 mg/kg/day dosage, the small increase in relative liver weight in male rats was at this dosage not considered evidence for an adverse effect on the liver. Results of the 4-week gavage study in rats were similar to those of the 13-week study, identifying no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values of 250 and 750 mg/kg/day, respectively, for liver effects. Observed effects consistent with hepatotoxicity included increased absolute and relative liver weights (≥250 mg/kg/day in males and 750 mg/kg/day in females), increased incidence of hepatocyte centrilobular (≥250 mg/kg/day in males and 750 mg/kg/day in females), and increase serum liver enzyme activity (alanine aminotransferase) (750 mg/kg/day in males and females). Histopathological changes characterized by cloudy swelling of parenchymal cells of the liver and an increase in liver weight were observed in female rats administered 408 mg/kg/day by gavage for 6 months. No other hepatic changes were reported. No liver effects were observed in female rats administered 136 mg/kg/day. However, this study was poorly reported and did not provide adequate descriptions of study methods or results.

Guinea pigs exposed to sublethal concentrations of ethylbenzene (≤10,000 ppm for <100 minutes) showed “moderate” pulmonary edema and congestion. These findings had disappeared in animals after a 4–8-day recovery period, suggesting that these pathological effects in the lung are reversible. A 50% respiratory depression was observed in mice exposed to ≥1,432 ppm for 5–30 minutes. Respiratory depression has not been reported in humans exposed to ethylbenzene. Nasal and eye irritation was evident in animals exposed to 1,000 ppm for ≥3 minutes. An NTP study did not observe weight or histopathological effects in the lungs of rats or mice exposed to 782 ppm or rabbits exposed to 1,610 ppm ethylbenzene for 4 weeks. Absolute and relative lung weight was increased in rats, but not mice, exposed to ≥250 ppm for 13 weeks; no treatment-related histopathological effects were observed. Another study did not report pulmonary injury in rats, guinea pigs, rabbits, or monkeys exposed to 600–2,200 ppm ethylbenzene for approximately 6 months; however, only two animals were used in some of the dose groups in rabbits and monkeys. In the NTP study, no treatment-related histopathological effects were
noted in respiratory tissue in rats or female mice exposed to up to 750 ppm ethylbenzene for 2 years. Although an increase in alveolar epithelial hyperplasia was noted in male mice in the 750-ppm group the incidence fell within historical controls for the conducting laboratory. The available data on adverse respiratory effects associated with ethylbenzene exposure in animals and the limited data available in humans suggest that respiratory effects in humans could result following inhalation exposure to high concentrations of ethylbenzene. Respiratory effects from low-level exposure, such as that found in the outdoor air, appear to be less likely.

Developmental effects have been reported in the offspring of pregnant animals exposed to ethylbenzene during gestation. The best reported studies available suggest that developmental effects are generally observed at concentrations of approximately ≥1,000 ppm. Significant increases in the incidence of fetal skeletal variations were observed in the offspring of pregnant rats exposed to 2,000 ppm and reductions in fetal body weight were observed in the offspring of pregnant rats exposed to ≥1,000 ppm ethylbenzene during gestation. Maternal toxicity, manifested as reduced body weight gain, was also observed in rats exposed to ≥1,000 ppm. No developmental effects were observed at concentrations of ≤500 ppm. In contrast, an increased incidence of fetuses with extra ribs was observed in the offspring of rats exposed to 100 ppm during gestation, but not when the animals were exposed to 100 ppm during pre-mating and gestation. No other significant increases in major malformations or minor anomalies were observed. Neurodevelopmental assessments conducted on F2 rat offspring indicated no effects in a functional observational battery assessment, fore- or hind-limb grip strength, swimming ability, motor activity, startle response, or learning and memory assessments at 500 ppm.

The number of implantations or live fetuses per litter and the percentage of resorptions or non-live implants per litter were unaffected in pregnant rats exposed to 2,000 ppm ethylbenzene during gestation. In a two-generation study, estrous cycle length was significantly reduced in F0, but not F1, females exposed to 500 ppm or in rats or mice exposed to 975 ppm ethylbenzene for 90 days. Reproductive parameters were not affected in F0 or F1 males or females at 500 ppm ethylbenzene. Exposure of rats and rabbits to 100 or 1,000 ppm ethylbenzene for 3 weeks during prior to mating or gestation or both resulted in no conclusive evidence of reproductive effects in either species. Assessments of reproductive organs conducted following intermediate- and chronic-duration exposure to ethylbenzene have not observed histopathological changes in the testes of rats, mice, or rabbits exposed to concentrations as high as 2,400 ppm ethylbenzene for 4 days or in rats or mice exposed to 782 ppm ethylbenzene or rabbits exposed to 1,610 ppm for 4 weeks. No effect was observed on spermatid counts, sperm motility, weight of the caudal epididymis, or testicular morphology in rats or mice exposed to 975 ppm ethylbenzene for
Other systemic effects have been observed in animals after acute-, intermediate-, and chronic-duration exposures to ethylbenzene. Eye irritation and lacration have been observed after acute-duration exposures in rats, mice, and guinea pigs exposed to \( \geq 1,000 \) ppm ethylbenzene. Lacration was observed in rats exposed to 382 ppm for 4 weeks. In contrast, no ocular effects were seen in rats or mice after a 13-week exposure to 975 ppm ethylbenzene. Mild irritation, reddening, exfoliation, and blistering have been reported in rabbits when ethylbenzene was applied directly on the skin. Slight irritation of the eye and corneal injuries were observed in rabbits when ethylbenzene was instilled onto the eyes.

One study examined the possible association between occupational exposure to ethylbenzene and increased cancer risk; no cases of malignancy were observed in workers exposed to ethylbenzene for 20 years. Animal studies have found increased incidences of neoplasms in rats and mice following inhalation or oral exposure, which are considered relevant to humans. The inhalation studies conducted by NTP found clear evidence of carcinogenic activity in male rats based on increased incidences of renal tubule neoplasms and testicular adenomas, some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenomas, some evidence of carcinogenic activity in male mice based on increased incidences of alveolar/bronchiolar neoplasms, and some evidence of carcinogenic activity in female mice based on increased incidences of hepatocellular neoplasms. In a reevaluation of the histopathology of rat kidneys from the NTP study, another study confirmed the NTP findings and suggested that the increased incidence of kidney tumors in rats in the high-dose group was related to a chemical-induced exacerbation of chronic progressive nephropathy (CPN) with a minor contributing factor in male rats being \( \alpha_2 \)-globulin nephropathy. The author suggests that since CPN is an age-related disease of rodents without a counterpart in humans, the kidney results of the NTP study are not relevant to humans for risk assessment purposes. However, in an analysis of the association between CPN and renal tubule cell neoplasms in male F344 rats, it was concluded that the association between CPN and renal tubule cell neoplasms is marginal. Results of this analysis suggest that the number of renal tubule cell neoplasms secondary to CPN would be few. An increase in the total number of malignant tumors was observed in rats orally exposed to ethylbenzene; however, data on specific tumor types were not provided. On the basis of the NTP study, IARC has classified ethylbenzene as a Group 2B carcinogen (possibly carcinogenic to humans). In the most recent carcinogenicity assessment by the EPA conducted in 1991, ethylbenzene was classified as Group D (not classifiable as to human carcinogenicity) due to the lack of
2. RELEVANCE TO PUBLIC HEALTH

animal bioassays and human studies; however, the EPA assessment predated the NTP study. Ethyl-
benzene is not included in the DHHS’s 11th Report on Carcinogens.

Acute- and intermediate-duration studies provide strong evidence that ototoxicity is a sensitive effect following inhalation exposure to ethylbenzene. A more detailed discussion of this effect follows. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on other effects.

A study of workers exposed occupationally to solvent mixtures that include ethylbenzene (mean exposure level 1.8 ppm) showed a 58% incidence of hearing loss compared to 36% in the reference (unexposed) group. The role of ethylbenzene in the observed losses cannot be ascertained from this study given that ethylbenzene was only one of several solvents, most of which were present at mean concentrations 1.5–3.5 times higher than ethylbenzene. Consistent with the outcome of occupational studies showing hearing loss, significant and persistent adverse auditory effects have been shown in animals after acute- and intermediate-duration inhalation exposures to ethylbenzene and after acute-duration oral exposures. OHCs in the organ of Corti (located in the cochlea) are a sensitive target of toxicity of ethylbenzene. Significant losses of OHCs in the organ or Corti were observed in male rats after acute-duration inhalation exposure to ≥400 ppm and intermediate-duration inhalation exposure to ≥200 ppm ethyl-
benzene. These losses in OHC were observed 8–11 weeks after the last exposures. Inhalation of ≥400 ppm ethylbenzene for 5 days or 4 weeks also resulted in a significant deterioration of auditory thresholds. The magnitude of the shifts in auditory thresholds observed after the first 4 weeks of exposure did not change during a 13-week exposure period or after an 8-week post-exposure recovery period. Inner hair cells were affected by ethylbenzene only at ≥600 ppm in the intermediate-duration study. Guinea pigs exposed to ethylbenzene at 2,500 ppm for 5 days did not show auditory deficits or losses in outer hair cells, whereas significant deficits and hair cell loss were observed in rats exposed to ethyl-
benzene at 550 ppm. An almost complete loss of OHC was reported in male rats 10 days after an acute-duration oral exposure to ethylbenzene. The mechanisms of the species differences between rats and guinea pigs are not understood. However, given the observations of hearing loss in workers exposed to 1.8 ppm ethylbenzene, the rat appears to be an appropriate animal model.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for ethylbenzene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an
appreciable risk of adverse effects (noncancerogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

**Inhalation MRLs**

- An MRL of 5 ppm has been derived for acute-duration inhalation exposure (14 days or less) to ethylbenzene.

There is limited information on the acute toxicity of ethylbenzene in humans. Acute exposures to \( \geq 1,000 \) ppm resulted in ocular irritation, a burning sensation, and profuse lacrimation (Cometto-Muniz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930). Volunteers exposed to 2,000 ppm reported irritation and chest constriction with worsening symptoms when the concentration was increased to 5,000 ppm (Yant et al. 1930). Studies in laboratory animals identify ototoxicity as the most sensitive end point for acute-duration inhalation exposure to ethylbenzene. Damage to the outer hair cells (OHCs) of the organ of Corti and, in some cases, significant reductions in auditory thresholds were observed in rats exposed to \( \geq 400 \) ppm ethylbenzene by inhalation for 5 days (Cappaert et al. 1999, 2000, 2001, 2002). Loss of OHCs appeared to be concentration-related as losses were 52–66% in animals exposed to 800 ppm ethylbenzene (Cappaert et al. 1999), 40–75% at 550 ppm, and approximately 25% at 400 ppm (Cappaert et al. 2000, 2001). OHC losses in rats exposed to 300 ppm were small (12%) and not statistically significant (Cappaert et al. 2000). Auditory thresholds in rats exposed to ethylbenzene at \( \geq 400 \) ppm were significantly affected in the mid-frequency region; however, an increasingly broader range of frequencies was affected with increasing concentrations of ethylbenzene (Cappaert et al. 1999, 2000). Auditory assessments indicate that effects were evident shortly after exposure and persisted for up
2. RELEVANCE TO PUBLIC HEALTH

to 11 weeks (termination of the observation period) (Cappaert et al. 1999, 2000, 2001, 2002), suggesting that the auditory effects might be irreversible. Cappaert et al. (2002) demonstrated a significant species difference in the susceptibility of rats and guinea pigs to the ototoxic effects of ethylbenzene, with guinea pigs showing no auditory deficits or losses in OHCs at 2,500 ppm ethylbenzene after 5 days (Cappaert et al. 2002).

Neurological effects were observed after acute-duration exposure to ethylbenzene at concentrations equal to or higher than those that elicited auditory effects in animals. Effects observed after acute-duration exposure to ethylbenzene include moderate activation of motor behavior in rats exposed to 400 ppm (Molnar et al. 1986) and reduced activity and prostration and shallow breathing in rats and mice at 1,200 ppm (Ethylbenzene Producers Association 1986a). Rats or mice exposed to ≥2,000 ppm showed posture changes, reduced grip strength, reduced motor coordination (Tegeris and Balster 1994), narcotic effects (Molnar et al. 1986), and neurotransmission disturbances in the forebrain and hypothalamus (Andersson et al. 1981). Mice exposed to 4,060 ppm for 20 minutes showed a 50% reduction in respiratory rate (Nielsen and Alarie 1982). A 50% respiratory depression observed in mice at 1,432 ppm was attributed to sensory irritation (De Ceaurrez et al. 1981).

Increased liver weight was reported after acute-duration exposure in rats exposed to ≥400 ppm ethylbenzene (Ethylbenzene Producers Association 1986a; Toftgard and Nilsen 1982), but not in mice at 1,200 ppm or rabbits at 2,400 ppm (Ethylbenzene Producers Association 1986a). At these same levels and exposure durations, induction of microsomal enzymes and related ultrastructural changes (e.g., proliferation of the smooth endoplasmic reticulum) were observed. These effects occurred in the absence of histopathological changes to the liver. Therefore, the effects on the liver appear to be related to induction of microsomal enzymes in smooth endoplasmic reticulum. An increase in relative kidney weight was also observed in rats exposed to ≥1,200 ppm (Ethylbenzene Producers Association 1986a; Toftgard and Nilsen 1982), but not in mice at 1,200 ppm or rabbits at 2,400 ppm (Ethylbenzene Producers Association 1986a). However, increased kidney weights occurred in the absence of histological changes (Ethylbenzene Producers Association 1986a). No histopathological alterations were observed in the lungs of surviving rats, mice, or rabbits exposed to 1,200, 400, or 2,400 ppm ethylbenzene, respectively, for 4 days (Ethylbenzene Producers Association 1986a).

The observed damage to the auditory capacity of rats exposed to ethylbenzene during acute-duration studies reported in Cappaert et al. study (2000) was chosen as a critical effect to derive the acute-duration inhalation MRL. In the study by Cappaert et al. (2000), Wag/Rij rats (8 rats/group; sex not provided)
were exposed to 0, 300, 400, or 550 ppm ethylbenzene (99% pure) 8 hours/day for 5 days. Potential auditory effects were examined by measuring distortion product otoacoustic emissions (DPOAE), compound action potential (CAP), and hair cell counts at five locations of the organ of Corti 3–6 weeks after the last ethylbenzene exposure. Exposed animals did not show clinical signs of intoxication and there were no significant differences in terminal body weight between exposed and control rats. DPOAE amplitude growth curves showed a significant reduction in rats exposed to 550 ppm, but not to 300 or 400 ppm ethylbenzene. Effects were significant at 5.6, 8, and 11.3 kHz, but not at other frequencies. The DPOAE thresholds were significantly shifted (increased stimulus was needed to elicit the threshold response) at 5.6, 8, 11.3, and 16 kHz in rats in the 550-ppm group. DPOAE threshold shifts were not observed in other exposure groups. Animals exposed to 550 ppm showed a significant shift in the CAP amplitude growth curves at 8, 12, and 16 kHz. In the 400-ppm group, the CAP growth curves were affected only at 12 kHz and there was no effect in animals in the 300-ppm group. CAP thresholds were significantly shifted at 8, 12, and 16 kHz in the 550-ppm group and at 12 and 16 kHz in the 400-ppm group. There was no significant deterioration of CAP thresholds in the 300-ppm group. Significant OHC losses of approximately 33 and 75% were observed in the 550-ppm group in the auditory regions corresponding to 11 and 21 kHz, respectively. In the 400-ppm group, significant losses (25%) were observed in the 11 kHz region. OHC losses in the 21 kHz region in the 300-ppm group were approximately 12%, but were not statistically significantly different from controls. This study identifies a NOAEL of 300 ppm and a LOAEL of 400 ppm for significant deterioration in CAP auditory thresholds and significant OHC losses.

The point of departure for an acute-duration inhalation MRL was identified using benchmark dose (BMD) analysis of the CAP auditory threshold data using data from this study provided by Dr. Cappaert to ATSDR. The largest effects on CAP threshold occurred in response to 8, 12, and 16 kHz stimuli and, on this basis, these data were selected for BMD modeling. The BMD modeling was run using an internal dose metric (time-averaged arterial blood concentration of ethylbenzene, MCA), which was simulated using a physiologically-based pharmacokinetic (PBPK) model. Using MCA as the dose metric, the CAP threshold shift data were fit to all available continuous models in EPA’s Benchmark Dose Software (BMDS, version 2.1.1) using a benchmark dose response (BMR) of 1 standard deviation. Details of the BMD modeling and PBPK models are present in detail in Appendix A. At the 8 kHz frequency, the Hill, polynomial (2- and 3-degree), and power models provided adequate fit to the data. Of these models, the polynomial (3-degree) model provided the best fit to the data. At 12 and 16 kHz frequencies, only the Hill model provided an adequate fit to the data. The 95% lower confidence limit on the benchmark concentration (BMDL_{MCA}) for these three models are presented in Table 2-1.
2. RELEVANCE TO PUBLIC HEALTH

Table 2-1. Human Equivalent Concentrations (HECs) for CAP Threshold Shifts

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model</th>
<th>BMCL&lt;sub&gt;MCA&lt;/sub&gt; (µmol/L)</th>
<th>HEC&lt;sup&gt;a&lt;/sup&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP threshold shift at 8 kHz</td>
<td>Polynomial (3-degree)</td>
<td>102.63</td>
<td>178.52</td>
</tr>
<tr>
<td>CAP threshold shift at 12 kHz</td>
<td>Hill</td>
<td>89.47</td>
<td>163.80</td>
</tr>
<tr>
<td>CAP threshold shift at 16 kHz</td>
<td>Hill</td>
<td>81.10</td>
<td>154.26</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated using a reference human body weight of 70 kg and the assumption of 14-day continuous exposure.

BMCL<sub>MCA</sub> = 95% lower confidence limit on the benchmark concentration associated with a benchmark response of 1 standard deviation estimated using an MCA (time-weighted arterial blood concentration of ethylbenzene) dose metric; CAP = compound action potential
To select the point of departure for the acute-duration inhalation MRL, human equivalent concentrations (HECs) were predicted from the BMCL\textsubscript{MCA} values in Table 2-1 for CAP threshold data at 8, 12, and 16 kHz using the human PBPK model (estimation of the HEC values is described in Appendix A). The lowest HEC of 154.26 ppm was selected as the point of departure. This HEC was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) resulting in an acute-duration inhalation MRL of 5 ppm.

- An MRL of 2 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to ethylbenzene.

Several studies in animals, but no studies in humans, have examined the toxicity of ethylbenzene following intermediate-duration inhalation exposure. The available animal studies suggest that ototoxicity is the most sensitive end point of ethylbenzene toxicity. Rats exposed to ≥400 ppm ethylbenzene via inhalation for 4 or 13 weeks showed significant increases in auditory thresholds. These threshold shifts persisted unchanged for the duration of the exposure period and during an 8-week post-exposure recovery period (Gagnaire et al. 2007). Cell counts conducted in the organ of Corti after the 8-week recovery period showed significant losses of outer hair cells in rats exposed to ≥200 ppm. Concentration-related losses of inner hair cells (IHC) (14 and 32%) were observed in animals in the 600 and 800 ppm groups, respectively, with occasional IHC losses in the 400 ppm group.

Systemic effects have been observed at concentrations equal to or higher than those that elicited ototoxic effects in rats. Increased liver, kidney, lung, and spleen weights have been observed in animals exposed to ethylbenzene concentrations in the 250–1,000 ppm range (Cragg et al. 1989; Elovaara et al. 1985; NIOSH 1981; NTP 1992; Wolf et al. 1956). However, the changes in organ weight have not been associated with histological alterations. One study (Cragg et al. 1989) reported a small, but statistically significant, increase in platelet counts in male rats and leukocyte counts in female rats exposed to 782 ppm ethylbenzene for 4 weeks. Developmental effects have been observed in rats exposed to ≥1,000 ppm. Increases in the occurrence of skeletal malformations (NIOSH 1981; Saillenfait et al. 2003) and decreases in fetal body weight (Saillenfait et al. 2003, 2006, 2007) have been observed at ≥1,000 ppm. The NOAEL for these effects is 500 ppm (NIOSH 1981; Saillenfait et al. 2003, 2006, 2007). Developmental landmarks and neurodevelopment were not statistically or biologically significantly affected in the offspring of rats exposed to up to 500 ppm ethylbenzene in a two-generation reproductive toxicity study (Faber et al. 2006, 2007).
Thus, the available intermediate-duration inhalation studies suggest that ototoxicity is the most sensitive effect of ethylbenzene. Ototoxicity observed in the study by Gagnaire et al. (2007) was selected as the critical effect to derive the intermediate-duration inhalation MRL. In the Gagnaire et al. (2007) study, male Sprague-Dawley rats (14 rats/exposure group) were exposed to 0, 200, 400, 600, and 800 ppm ethylbenzene (99% pure) 6 hours/day, 6 days/week for 13 weeks. Ototoxicity was assessed based on effects on neurophysiological measurements after 4, 8, or 13 weeks of exposure and after a 8-week recovery period and cochlear total hair cell counts were measured at the end of the 8-week recovery period. There were no significant differences in body weight gain between the surviving treated animals and controls. Audiometric thresholds at 2, 4, 8, and 16 kHz were significantly higher in animals exposed to 400, 600, and 800 ppm ethylbenzene than in controls. The effect was evident at week 4, did not change throughout the exposure period, and was not reversed after 8 weeks of recovery. No shift in audiometric thresholds was observed in rats in the 200 ppm group; however, the morphological assessment of the organ of Corti showed significant losses (up to 30% of the outer hair cells in the mid frequency region) in the third row of the OHC in four of eight rats exposed to 200 ppm. A concentration-related loss in third row OHC (OHC3) was evident with almost complete loss observed in the 600 and 800 ppm groups. The data suggest that the extent of the damage at each concentration was greatest in the OHC3 followed, in decreasing order, by damage in OHC2, OHC1, and IHC. There was no significant hair cell loss in the control animals.

Auditory threshold shifts and OHC loss are selected as the critical effects following intermediate-duration inhalation exposure to ethylbenzene. BMD analysis was conducted for these end points using a data set provided to ATSDR by Dr. Gagnaire. The data for auditory thresholds at 4, 8, and 16 kHz in rats exposed for 4 or 13 weeks and OHC loss at the end of the recovery period were fit to all available continuous models in EPA’s BMDS (version 2.1.1) using a time-averaged arterial blood concentration of ethylbenzene (MCA) as the dose metric and a BMR of 1 standard deviation change from control. The BMD modeling and the PBPK model used to estimate the MCA dose metric are described in Appendix A.

Most of the available BMD models did not adequately fit the auditory threshold data; however, with the exception of the auditory thresholds at 16 kHz in rats exposed to ethylbenzene for 13 weeks, one BMD model fit each data set often when the highest two dose groups were dropped. The BMCLMCA values estimated from these models are presented in Table 2-2. None of the BMD models adequately fit the OHC loss data. HECs corresponding to specific BMCLMCA values were simulated using a human PBPK model (described in Appendix A). The HECs of the BMCLMCA values (Table 2-2) ranged from 63.64 to
### Table 2-2. Human Equivalent Concentrations (HECs) for Auditory Effects in Sprague-Dawley Rats Exposed to Ethylbenzene 6 Hours/Day, 6 Days/Week for 4 or 13 Weeks

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model</th>
<th>BMCL&lt;sub&gt;MCA&lt;/sub&gt; µmol/L</th>
<th>HEC&lt;sup&gt;a&lt;/sup&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory thresholds at 4 kHz following 4 weeks of exposure</td>
<td>Power (two highest doses dropped); nonconstant variance</td>
<td>33.12</td>
<td>87.13</td>
</tr>
<tr>
<td>Auditory thresholds at 8 kHz following 4 weeks of exposure</td>
<td>2-Degree polynomial (two highest doses dropped); nonconstant variance</td>
<td>19.94</td>
<td>63.64</td>
</tr>
<tr>
<td>Auditory thresholds at 16 kHz following 4 weeks of exposure</td>
<td>Power (two highest doses dropped); nonconstant variance</td>
<td>27.31</td>
<td>77.77</td>
</tr>
<tr>
<td>Auditory thresholds at 4 kHz following 13 weeks of exposure</td>
<td>Hill (all doses); nonconstant variance</td>
<td>28.59</td>
<td>79.95</td>
</tr>
<tr>
<td>Auditory thresholds at 8 kHz following 13 weeks of exposure</td>
<td>Power (two highest doses dropped); nonconstant variance</td>
<td>29.71</td>
<td>81.79</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated using a reference human body weight of 70 kg and the assumption of 364-day continuous exposure.

BMCL<sub>MCA</sub> = 95% lower confidence limit on the benchmark concentration associated with a benchmark response of 1 standard deviation estimated using an MCA (time-weighted arterial blood concentration of ethylbenzene) dose metric.
2. RELEVANCE TO PUBLIC HEALTH

87.13 ppm; the lowest HEC of 63.64 ppm was selected as the point of departure for the MRL. This HEC was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) resulting in an intermediate-duration inhalation MRL of 2 ppm.

- An MRL of 0.06 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to ethylbenzene.

The chronic toxicity of inhaled ethylbenzene has been examined humans and in 2-year bioassays in rats and mice (NTP 1999). Hematological effects (increased average number of lymphocytes and decreased hemoglobin) were observed in workers exposed to solvents containing ethylbenzene (Angerer and Wulf 1985). In rats, concentration-related increases in the severity of nephropathy were observed in female rats exposed to ≥75 ppm and in male rats exposed to 750 ppm (NTP 1999). Increases in the incidence of renal tubule hyperplasia were also observed in male and female rats exposed to 750 ppm. The lowest LOAEL identified in mice was 250 ppm for hyperplasia of pituitary gland pars distalis observed in females; at 750 ppm, thyroid follicular cell hyperplasia was observed in male and female mice and hypertrophy and necrosis of the liver were observed in male mice (NTP 1999). These studies identify the kidney as a sensitive target following chronic-duration inhalation exposure to ethylbenzene and the NTP (1999) rat study was selected as the basis of the MRL.

In the NTP (1999) study, groups of male and female F344/N rats (50 animals/sex/dose group) were exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation 6 hours/day, 5 days/week, for 104 weeks. Animals were observed twice daily and clinical findings were recorded monthly. Body weights were measured throughout the study and a complete necropsy and microscopic examination of major tissues and organs were performed on all rats. Survival of male rats in the 750 ppm group was significantly less than that of the chamber controls. No clinical findings were attributed to ethylbenzene exposure. Although the incidence of nephropathy (47/50, 43/50, 47/50, and 48/50 in males and 38/50, 42/50, 43/50, 46/49 in females) was not significantly different between the groups, significant increases in the severity of the nephropathy were observed in females at ≥75 ppm and in males at 750 ppm. The nephropathy severity scores in the 0, 75, 250, and 750 ppm groups were 2.3, 2.4, 2.3, and 3.5 in males, respectively, and 1.3, 1.6, 1.7, and 2.3 in females, respectively. Additionally, a significant increase in the incidence of renal tubule hyperplasia was observed in male rats exposed to 750 ppm. The incidences of renal tubule adenoma and adenoma or carcinoma (combined) in the 750-ppm group were significantly greater than the incidence in control animals. An increase in the incidence of cystic degeneration of the liver was also observed in male rats at 750 ppm.
Increased severity of chronic progressive nephropathy observed in female rats exposed to ≥75 ppm (NTP 1999) was selected as the critical effect for the MRL. BMD analysis was considered for determining the point of departure for the MRL; however, none of the available continuous exposure BMD models fit the data (standard errors calculated using the raw severity score data). Thus, a NOAEL/LOAEL approach was selected for calculating the point of departure; application of BMD analysis was precluded because standard errors or standard deviations were not provided for the nephropathy severity ratings. A PBPK model was developed (see Appendix A for details) to simulate two internal dose metrics for kidney effects: time-averaged arterial blood concentration of ethylbenzene (MCA) and time-averaged rate of metabolism of ethylbenzene expressed per kg body mass (MRAMKB). Both metrics were explored because current knowledge of the mechanisms of toxicity of ethylbenzene does not include an understanding of the relative contribution of parent compound or metabolites as proximate toxic agents in kidney. The internal dose metrics (MCA and MRAMKB) for each exposure level are presented in Table 2-3.

Human equivalent concentrations (HECs) corresponding to LOAELMCA and LOAELMRAMKB in female rats was estimated using a human PBPK model (described in Appendix A). The HECs were 17.45 ppm for the MCA dose metric and 52.68 ppm for the MRAMKB dose metric. Because there is limited information to determine whether the observed renal toxicity in female rats exposed to ethylbenzene is due to ethylbenzene or its metabolites, the lowest HEC value (17.45 ppm) was selected as the point of departure for the MRL. The HECMCA of 17.45 ppm was divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability) resulting in a chronic-duration inhalation MRL of 0.06 ppm.

**Oral MRLs**

No studies describing acute-duration oral exposure of humans to ethylbenzene were found in the literature. Two animal studies have examined the acute oral toxicity of ethylbenzene. An almost complete loss of the three rows of OHCs in the organ of Corti were reported in male rats administered 900 mg/kg/day (the only dose tested) by gavage for 2 weeks (Gagnaire and Langlais 2005). These losses were observed 10 days after the last dose. Although losses of OHCs have also been observed in acute-duration inhalation studies (Cappaert et al. 1999, 2000, 2001, 2002), Gagnaire and Langlais (2005) did not have a control group to establish the magnitude of the effects relative to unexposed animals; the study was used to rank the ototoxicity of 21 solvents administered by gavage. Nevertheless, the OHC losses observed in the ethylbenzene-treated animals were among the highest observed among the 21 organic
### Table 2-3. Internal Dose Metrics for Male and Female F344/N Rats Exposed to Ethylbenzene 6 Hours/Day, 5 Days/Week for 104 Weeks

<table>
<thead>
<tr>
<th>Exposure level (ppm)</th>
<th>Arterial ethylbenzene concentration (MCA, µmol/L)</th>
<th>MRAMKB (µmol/hour/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>4.12</td>
<td>8.92</td>
</tr>
<tr>
<td>250</td>
<td>27.66</td>
<td>23.64</td>
</tr>
<tr>
<td>750</td>
<td>146.77</td>
<td>43.05</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>4.16</td>
<td>10.00</td>
</tr>
<tr>
<td>250</td>
<td>28.72</td>
<td>26.04</td>
</tr>
<tr>
<td>750</td>
<td>150.68</td>
<td>46.49</td>
</tr>
</tbody>
</table>

*Time weighted average body weight of 0.43 kg.

*Time weighted average body weight of 0.27 kg.

MCA = time-averaged arterial blood concentration; MRAMKB = time averaged rate of ethylbenzene metabolism expressed per kg body mass.
2. RELEVANCE TO PUBLIC HEALTH

solvents tested (Gagnaire and Langlais 2005). The 900 mg/kg/day dose was considered a serious LOAEL for ototoxicity. In the second study, doses of 500 or 1,000 mg/kg ethylbenzene decreased luteinizing hormone, progesterone, and 17 β-estradiol levels, increased stromal tissue with dense collagen bundles and reduced lumen in the uterus, and delayed the estrus cycle in female rats during the diestrus stage (Ungvary 1986). Interpretation of the results of this study is limited by the poor reporting of the study methods and results and the lack of statistical analysis. Because the only dose tested in the Gagnaire and Langlais (2005) study is a serious LOAEL, an acute-duration oral MRL cannot be derived for ethylbenzene.

- An MRL of 0.4 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to ethylbenzene.

The intermediate-duration oral database for ethylbenzene is limited to a study conducted by Mellert et al. (2007) evaluating the effects of oral exposure of rats to ethylbenzene for 4 and 13 weeks, and a poorly reported 6-month exposure study in rats (Wolf et al. 1956). The 4- and 13-week studies by Mellert et al. (2007) found effects consistent with hepatotoxicity including increased absolute and relative liver weights, increased incidence of hepatocyte centrilobular hypertrophy, and increased serum liver enzyme activities in rats administered ≥250 mg/kg/day. Kidney effects, including increases in increases in relative kidney weight and hyaline droplet nephropathy were observed in males administered ≥250 mg/kg/day; however, these effects were most likely secondary to increases of α2µ-globulin accumulation, and, therefore, considered not relevant to humans. Wolf et al. (1956) also reported liver effects (characterized by cloudy swelling of parenchymal cells of the liver and an increase in liver weight) in female rats administered 408 mg/kg/day by gavage for 6 months. No other hepatic changes were reported. However, this study was poorly reported and did not provide adequate descriptions of study methods or results.

Based on evidence of hepatotoxicity (increased serum liver enzyme activity, absolute and relative liver weights, dose-related increased incidence of centrilobular hepatocyte hypertrophy), and the lack of evidence for adverse effects in other tissues or organ systems at lower oral intermediate-duration dosages, liver effects were selected as the basis for deriving the intermediate oral MRL. The principal study (Mellert et al. 2007) identified NOAEL and LOAEL values for hepatotoxicity of 75 and 250 mg/kg/day, respectively, in rats administered ethylbenzene for 13 weeks. In this study (Mellert et al. 2007), groups of 10 male and 10 female Wister rats were administered ethylbenzene (no vehicle) by gavage at doses of 0, 75, 250, or 750 mg/kg/day for 13 weeks. The total daily dose of ethylbenzene was administered as split morning/evening half doses. Animals were examined daily for mortality and clinical signs and food and
water consumption and body weights were recorded weekly. A detailed clinical examination
(ophthalmology and a functional observational battery [FOB]) and assessment of motor activity were
conducted during the last week of treatment. After 13 weeks, urinalysis was conducted and blood
samples were obtained and analyzed for hematology and clinical chemistry; organ weights were recorded
and gross histopathologic examinations of the liver, kidney, and pancreas were conducted on animals in
all groups. A comprehensive histopathological examination of tissues was performed in the control and
750 mg/kg/day groups. No mortalities were observed during the course of the study. Clinical signs (post­
dosing salivation) in treated animals were observed in all animals administered ≥250 mg/kg/day and in
one animal administered 75 mg/kg/day. Terminal body weight in males was significantly decreased by
14% compared to controls in the 750 mg/kg/day group. Mean corpuscular volume was increased in males
and females and platelet count was reduced in females treated with 750 mg/kg/day. Prothrombin time
was significantly decreased (<8% compared to controls) in females administered ≥250 mg/kg/kg, but no
changes in prothrombin times were observed in males in any treatment group. Effects indicative of liver
toxicity (summarized in Table 2-4) included increased activity of serum liver enzymes (alanine
aminotransferase and γ-glutamyl transferase), increased absolute and relative liver weights, and a dose­related increase in the incidence of centrilobular hepatocyte hypertrophy. Although some alterations in
other serum chemistry parameters were found, the study investigators considered them to be due to
adaptive effects in the liver.

Renal effects in males included increased serum creatinine (750 mg/kg/day), increased incidences of
transitional epithelial cells and granular and epithelial cell casts in the urine (≥250 mg/kg/day), increased
absolute and relative kidney weights (≥250 mg/kg/day), and a dose-related increase in severity of hyaline
droplet nephropathy (≥250 mg/kg/day). Adverse renal effects in males were most likely related to
accumulation of α2µ-globulin accumulation, and, therefore, considered not relevant to humans. Absolute
kidney weight was significantly increased by 7 and 13% in females administered 250 and 750 mg/kg/day,
respectively, compared to controls. However, since no histopathological findings or alterations in
urinalysis parameters were observed, increased kidney weight in females was not considered adverse.
Absolute and relative thymus weights were decreased in females treated with ≥250 mg/kg/day, but no
histopathological findings were observed. Histopathological examination of all other tissues did not
reveal any abnormalities. Results of the FOB did not reveal consistent treatment-related effects. NOAEL
and LOAEL values of 250 and 750 mg/kg/day, respectively, were identified based on hepatotoxicity in
male and female rats.
### Table 2-4. Effects on Serum Liver Enzymes, Liver Weights, and Liver Histopathology in Male and Female Rats Exposed to Oral Ethylbenzene for 13 Weeks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose group (mg/kg/day)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>75</td>
<td>250</td>
</tr>
<tr>
<td>ALT (µkat/L)</td>
<td>0.62±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70±0.12</td>
<td>0.89±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GGT (nkat/L)</td>
<td>2±3</td>
<td>6±6</td>
<td>10±6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute liver weight (g)</td>
<td>8.02±0.55</td>
<td>8.26±0.81</td>
<td>10.25±0.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver/body weight (%)</td>
<td>2.26±0.08</td>
<td>2.36±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.01±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Centrilobular hepatocyte hypertrophy (incidence)</td>
<td>1/10</td>
<td>1/10</td>
<td>6/10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean±standard deviation.

<sup>b</sup>p≤0.01.

<sup>c</sup>p≤0.05.

ALT = alanine aminotransferase; GGT = γ-glutamyl transferase

Source: Mellert et al. 2007
2. RELEVANCE TO PUBLIC HEALTH

Based on evidence of hepatotoxicity (increased serum liver enzyme activity, absolute and relative liver weights, and incidence of centrilobular hepatocyte hypertrophy), the liver was identified as the most sensitive target for oral ethylbenzene, with NOAEL and LOAEL values of 75 and 250 mg/kg/day, respectively. Since serum liver enzyme activities were increased in the 250 and 750 mg/kg/day groups in males, but only in the 750 mg/kg/day group in females, males appeared more sensitive than females to hepatic effects of oral ethylbenzene.

To determine the point of departure for derivation of the intermediate-duration MRL, data sets for serum liver enzymes (alanine aminotransferase and \(\gamma\)-glutamyl transferase), absolute liver weight, relative liver weight, and centrilobular hepatocyte hypertrophy in male rats were evaluated for suitability for BMD modeling using EPA’s BMDS (version 2.1.1). A PBPK model was developed to simulate two internal dose metrics for liver effects: time-averaged concentration of ethylbenzene in liver (MCL) and time-averaged rate of metabolism of ethylbenzene in liver (MRAMKL). The assumption of using the MCL metric is that the liver response is correlated with the time-averaged concentration of ethylbenzene in liver. The assumption in using the MRAMKL metric is that the liver response is correlated with the time-averaged rate of production of ethylbenzene metabolites in liver. Both metrics were explored because current knowledge of the mechanism of toxicity of ethylbenzene does not include an understanding of the relative contributions of parent compound or metabolites as proximate toxic agents in liver. Detailed discussions of the BMD modeling and PBPK model are presented in Appendix A.

Using the MCL and MRAMKL dose metrics, data for alanine aminotransferase, \(\gamma\)-glutamyl transferase, absolute liver weight, and relative liver weight were analyzed using all available continuous variable BMD models and a BMR of 1 standard deviation change from control and incidence data for centrilobular hepatocyte hypertrophy were analyzed using all available dichotomous BMD models and the extra risk option with a BMR of 10% extra risk. The BMD analysis identified nine BMDL values that could be used as the point of departure for the intermediate-duration oral MRL; these values are presented in Tables 2-5 and 2-6. For each end point and dose metric, human equivalent doses (HEDs) were estimated from the BMDL value using the human PBPK model (discussed in Appendix A); the HEDs are summarized in Tables 2-5 and 2-6.

The lowest HEDs were calculated from the BMDL\(_{MCL}\) and BMDL\(_{MRAMKL}\) for centrilobular hepatocyte hypertrophy. Because there is limited information to determine whether the observed hepatic effects are due to ethylbenzene or its metabolites, the lowest HED value (10.68 mg/kg/day) was selected as the point of departure for the MRL. The HED was divided by an uncertainty factor of 30 (3 for extrapolation from...
## 2. RELEVANCE TO PUBLIC HEALTH

Table 2-5. Benchmark Doses and Human Equivalent Doses for Liver Effects Using Arterial Ethylbenzene Concentration (MCL) Dose Metric

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model</th>
<th>BMDL (µmol/L)</th>
<th>HED(^a) (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased alanine aminotransferase</td>
<td>Hill (all doses); constant variance</td>
<td>7.49</td>
<td>11.82</td>
</tr>
<tr>
<td>Increased γ-glutamyl transferase</td>
<td>Inadequate fit to all models</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased absolute liver weights</td>
<td>Linear (highest dose dropped); constant variance</td>
<td>32.17</td>
<td>31.82</td>
</tr>
<tr>
<td>Increased relative liver weights</td>
<td>Linear (highest dose dropped); constant variance</td>
<td>13.53</td>
<td>18.47</td>
</tr>
<tr>
<td>Centrilobular hepatocyte hypotrophy</td>
<td>Log logistic</td>
<td>6.61</td>
<td>10.68</td>
</tr>
</tbody>
</table>

\(^a\)Calculated using a reference human body weight of 70 kg and the assumption that the daily dose was delivered in 16 dose splits/24 hours (i.e., only exposed during waking hours).

BMDL = 95% lower confidence limit on the benchmark dose; HED = human equivalent dose; MCL = time-averaged concentration of ethylbenzene in liver
**Table 2-6. Benchmark Doses (BMDs) and Human Equivalent Doses (HEDs) for Liver Effects Using MRAMKL Internal Dose Metric**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BMDL&lt;sup&gt;b&lt;/sup&gt; (µmol/hour/kg liver)</th>
<th>HED&lt;sup&gt;c&lt;/sup&gt; (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased alanine aminotransferase</td>
<td>3-Degree polynomial; nonconstant variance</td>
<td>391.02</td>
<td>31.06</td>
</tr>
<tr>
<td>Increased γ-glutamyl transferase</td>
<td>Linear; constant variance</td>
<td>737.62</td>
<td>111.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Increased absolute liver weights</td>
<td>Hill; constant variance</td>
<td>548.01</td>
<td>48.62</td>
</tr>
<tr>
<td>Increased relative liver weights</td>
<td>2-Degree polynomial; constant variance</td>
<td>390.47</td>
<td>31.01</td>
</tr>
<tr>
<td>Centrilobular hepatocyte hypertrophy</td>
<td>Multistage (3-degree polynomial)</td>
<td>206.91</td>
<td>15.48</td>
</tr>
</tbody>
</table>

<sup>a</sup>All doses used for BMD modeling.

<sup>b</sup>Calculated using a reference human body weight of 70 kg and the assumption that the daily dose was delivered in 16 dose splits/24 hours (i.e., only exposed during waking hours).

<sup>c</sup>Approximate value, value is very close to the metabolism Vmax.

BMDL = 95% lower confidence limit on the benchmark dose; MRAMKL = time-averaged rate of metabolism of ethylbenzene in liver
animals to humans using dosimetric adjustments and 10 for human variability), resulting in an intermediate-duration oral MRL of 0.4 mg/kg/day.

No studies describing the non-carcinogenic effects of chronic-duration oral exposure to ethylbenzene in humans were located. Available chronic-duration oral exposure studies in animals (Maltoni et al. 1985, 1997) did not evaluate comprehensive noncancer end points and, therefore, do not provide suitable data for derivation of a chronic-duration oral MRL.