Risk assessments for chronic exposure of children and prospective parents to ethylbenzene (CAS No. 100-41-4)

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Abstract
Potential chronic health risks for children and prospective parents exposed to ethylbenzene were evaluated in response to the Voluntary Children’s Chemical Evaluation Program. Ethylbenzene exposure was found to be predominately via inhalation with recent data demonstrating continuing decreases in releases and both outdoor and indoor concentrations over the past several decades. The proportion of ethylbenzene in ambient air that is attributable to the ethylbenzene/styrene chain of commerce appears to be relatively very small, less than 0.1% based on recent relative emission estimates. Toxicity reference values were derived from the available data, with physiologically based pharmacokinetic models and benchmark dose methods used to assess dose–response relationships. An inhalation non-cancer reference concentration or RfC of 0.3 parts per million (ppm) was derived based on ototoxicity. Similarly, an oral non-cancer reference dose or RfD of 0.5 mg/kg body weight/day was derived based on liver effects. For the cancer assessment, emphasis was placed upon mode of action information. Three of four rodent tumor types were determined not to be relevant to human health. A cancer reference value of 0.48 ppm was derived based on mouse lung tumors. The risk characterization for ethylbenzene indicated that even the most highly exposed children and prospective parents are not at risk for non-cancer or cancer effects of ethylbenzene.

Keywords
cancer, exposure assessment, hepatotoxicity, mode of action, ototoxicity

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Introduction
The Voluntary Children’s Chemical Evaluation Program (VCCEP) (US Environmental Protection Agency [EPA] 2000a) is a component of 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, 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) is a high production volume or HPV chemical, with production of 14,395 millions of pounds per year as of 2002 (Organisation for Economic Co-operation and Development [OECD] 2005). It is also a natural component of crude oil and refined petroleum products. Ethylbenzene was selected for the VCCEP Pilot because it was detected in human blood by the National Health and Nutrition Examination Survey (NHANES) (Ashley et al. 1994) and in expired air by the Total Exposure Assessment Methodology (TEAM) (Wallace et al. 1987b) monitoring programs, it was detected in environmental media, and hazard data are available (OECD 2005). In June 2001, the American Chemistry Council [ACC] Ethylbenzene Panel volunteered to participate in VCCEP and to sponsor a Tier I assessment of ethylbenzene, which was reviewed at a peer consultation in February 2007 and subsequently revised (ACC 2007, Toxicology Excellence for Risk Assessment [TERA] 2007). The purpose of this paper is to update the Tier I ethylbenzene assessment in accordance with new data and EPA guidance. Ethylbenzene has two distinct chains of commerce: the refinery chain associated with petroleum-derived products, and the ethylbenzene/styrene chain associated with the manufacture of ethylbenzene and styrene, and its presence in styrenic products. Ethylbenzene is present in gasoline at about 2% by weight, and is a component of hydrocarbon solvents, in particular commercial mixed xylene, which may contain 6–15% of ethylbenzene by volume. As part of these mixtures, ethylbenzene may be found in diluents in varnishes,
paints, and lacquers, including some consumer products, as well as in solvents used in the rubber and chemical manufacturing industries (Agency for Toxic Substances and Disease Registry [ATSDR] 1999). Ethylbenzene from these sources constitutes the refinery chain of commerce. Exposure to ethylbenzene 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 (ACC 2005).

The ethylbenzene industry is closely tied to the styrene industry, with nearly all ethylbenzene (approximately 99%) consumed internally for the manufacture of styrene (ATSDR 1999). Styrene is an intermediate in the production of a number of commercially important polymers and co-polymers (poly-styrene, styrene–butadiene rubber, styrene–butadiene latexes, acrylonitrile–butadiene–styrene and styrene–acrylonitrile, unsaturated polyester resins, and miscellaneous products) used to make a wide variety of products of industrial, consumer, and medical importance (ATSDR 1999, Styrene Information and Research Center [SIRC] 2011, Styrene Forum 2012). Commercially important styrenic polymers may contain residual amounts of ethylbenzene from the production process. This product source and market chain constitute the ethylbenzene/styrene chain of commerce.

The major findings of the Tier 1 VCCEP exposure assessment for ethylbenzene (ACC 2007) were

- The major source is automotive emissions (refinery chain of commerce);
- The major exposure route for all receptors is inhalation, with dietary intake relatively minor;
- The major exposure microenvironment is the home (residential indoor air); and
- The contributions of the ethylbenzene/styrene chain of commerce to total inhalation and dietary exposures are relatively very small.

When the Tier 1 VCCEP exposure assessment for ethylbenzene was prepared in the mid-2000s, it was recognized that personal exposure measurements, the most accurate estimate of integrated exposure for actual individuals, are usually higher than, and inconsistently related to, both indoor and outdoor air measurements. Since the only nationally representative data available were ambient monitoring data, the approach taken to estimate ethylbenzene concentrations in homes and other important indoor microenvironments was to apply indoor/outdoor ratios estimated from the literature to central tendency and upper-bound urban and rural outdoor air concentrations. In the past several years, multiple large studies examining indoor and personal exposures to ethylbenzene and other volatile organic compounds (VOCs) have been published, and the Centers for Disease Control (CDC) has added VOCs to the analytes biomonitored in the general US population in the continuous NHANES. The updated ethylbenzene exposure assessment described in this paper is based on a distribution of nationally representative personal exposure data collected in the NHANES VOCs Study (Jia et al. 2008c).

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 VCCEP Pilot Announcement (Table 1) and overall this information is of suitable quality to support human health hazard and risk assessments for children and prospective parents. Ethylbenzene has been previously evaluated by several organizations with respect to cancer classification and quantitation of non-cancer hazards (US EPA 1991, International Programme on Chemical Safety [IPCS] 1996, ATSDR 1999, International Agency for Research on Cancer [IARC] 2000, OECD 2005). 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 use in this assessment. In particular, the existing US EPA’s Integrated Risk Information System (IRIS) values (US EPA 1991) will be discussed in greater detail. Proposed toxicity reference values that reflect the current state of knowledge were developed for use in this assessment.

A brief hazard assessment is provided to acquaint the reader with the extent and nature of toxicity data available for ethylbenzene. Key endpoints and studies are discussed in greater detail in “Toxicity reference value derivation” section. Of particular note in this section are analyses of cancer modes of action (MOAs) that include the modified Hill Criteria as well as an assessment of human relevance. Ethylbenzene provided a unique opportunity to assess four different tumor types, each with a different potential MOA, using the framework devised by the US EPA and International Life Sciences Institute (ILSI) Risk Science Institute (RSI)/IPCS (Meek et al. 2003, US EPA 2005b, Boobis et al. 2006). Next, the results of the exposure assessment are provided. The “Risk characterization” section integrates the results from the two preceding sections, and “Conclusions/Discussion” from the foregoing sections are provided.

### Hazard assessment

The following provides a summary hazard characterization for ethylbenzene of each of the toxicity endpoints covered by VCCEP (US EPA 2000a), which includes acute toxicity, mutagenicity, systemic toxicity, developmental and reproductive toxicity, immunotoxicity, metabolism and

| Table 1. Endpoints for the VCCEP. |
|-------------------------------|
| Tier 1 | Tier 2 | Tier 3 |
| Acute toxicity | Subchronic toxicity | Neurotoxicity screening battery |
| Repeated dose toxicity with reproductive and developmental toxicity screens | Prenatal developmental toxicity, Reproductive and fertility effects | Carcinogenicity |
| Bacterial reverse mutation assay | Immunotoxicity | Developmental neurotoxicity |
| In vitro or in vivo chromosomal aberrations or in vivo micronucleus test | In vivo chromosomal aberrations or in vivo micronucleus test, Metabolism and pharmacokinetics | |
pharmacokinetics, carcinogenicity, neurotoxicity, and developmental neurotoxicity. These endpoints were discussed in greater detail in ACC (2007). Relevant ethylbenzene toxicological literature for the ACC (2007) document was identified via PubMed and Google Scholar searches, identification of additional studies referenced in extant reviews and regulatory documents, and direct communication with experts involved in ethylbenzene research. Since that time, the first author of this paper has continuously monitored PubMed for relevant new ethylbenzene publications via weekly PubMed alerts. Relevant English-language papers were retrieved and reviewed by the authors.

**Laboratory animal and in vitro data**

**Acute toxicity**

Animal data demonstrate that ethylbenzene has low acute oral toxicity (rat median oral lethal dose of 3.5–5.5 g/kg body weight (bwt); Wolf et al. 1956, Smyth et al. 1962). High doses or inhaled concentrations are required to produce neurological effects (e.g., for rats exposed for 4 h, 2180 parts per million (ppm) was required to produce minimal narcotic effects, Molnár et al. 1986) and death (median lethal concentration of 4000 ppm for a 4-h exposure of rats; Smyth et al. 1962). High vapor concentrations can be irritating to mucous membranes, and liquid can cause irritation to the skin and eyes (1000 ppm or higher exposures of guinea pigs; Yant et al. 1930).

**Genotoxicity**

Ethylbenzene has been extensively tested for toxicity to genetic material using nearly every available type of genetic toxicity test (reviewed in Henderson et al. 2007). Ethylbenzene is negative for genotoxicity in all *in vivo* studies that have been conducted and predominately negative for genotoxicity in *in vitro* studies (Table 2). The National Toxicology Program (NTP) reported a positive result for gene mutations in the mouse lymphoma assay; however, this only occurred at the highest non-lethal dose tested, and was accompanied by cytotoxicity (NTP 1999). NTP concluded that ethylbenzene gave little indication of mutagenicity in *in vitro* or *in vivo* (NTP 1999). Although ethylbenzene has not been evaluated specifically for germ cell mutations, its potential for producing a positive result is low given the weight of evidence in bacteria and somatic cell systems. 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 subchronic oral studies (NTP 1992a, 1999, Meller et al. 2004, 2007, Li et al. 2010). Overall, ethylbenzene is a moderate repeated exposure toxicity hazard with consistent targeted effects to the liver and kidney at concentrations ≥ 250 ppm or doses ≥ 250 mg/kg bwt/day.

Table 2. Summary of genotoxicity studies.

| In Vivo/In Vitro | Species (endpoint) | Exposure conditions | Results | Reference(s) |
|-----------------|-------------------|---------------------|---------|--------------|
| **In Vivo**     |                    |                     | Without metabolic activation | With metabolic activation | |
| 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 et al. (1985) |
| Human (sister chromatid exchange, DNA adducts, micronuclei, and strand breaks in lymphocytes) | Occupational exposure | – | NA | Holz et al. (1995) |
| **In Vitro**    |                    |                     |                     |                        |
| Mouse lymphoma (gene mutation) | Up to 120 µg/mL | – | – | Seidel et al. (2006) |
| Mouse lymphoma (gene mutation) | Up to 160 µg/mL | + | ND | NTP (1990) |
| Human lymphocytes (sister chromatid exchange) | Up to 1061.6 mg/L (+) | ND | NTP (1990) |
| Human lymphocytes (single strand breaks) | Up to 200 µM | + | NA | Chen et al. (2008) |
| Human lymphocytes (double-strand breaks) | 200 µM | – | NA | Chen et al. (2008) |
| Syrian hamster embryo cells (micronuclei) | 25 µg/ml | + | ND | Gibson et al. (1997) |
| Syrian hamster embryo cells (cell transformation) | 200 µg/ml | + | ND | Kerckaert et al. (1997) |
| **Salmonella** Typhimurium (gene mutation) | Up to 1,000 µg/plate | – | – | Zeiger et al. (1992) |
| **Salmonella** Typhimurium (gene mutation) | Up to 2000 µg/plate | – | – | Dean et al. (1985) |
| **Salmonella** Typhimurium (gene mutation) | Up to 3184 µg/plate | – | – | Florin et al. (1980) |
| **Salmonella** Typhimurium (gene mutation) | Up to 0.4 mg/plate | – | – | Nestman et al. (1980) |
| **Salmonella** Typhimurium (gene mutation) | Up to 1000 µg/plate | – | – | NTP (1999); NTP (1992a) |
| **Escherichia coli** (gene mutation) | Up to 2000 µg/plate | – | – | Dean et al. (1985) |
| **Saccharomyces cerevisiae** (gene mutation) | conc. not determined | – | – | Dean et al. (1985) |
| **S. cerevisiae** (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 et al. (1985) |
In subchronic inhalation studies, the liver and kidney effects were generally limited to increases in organ weights without the corresponding histopathological changes that occurred at ethylbenzene concentrations ≥ 250 ppm in rats and ≥ 750 ppm in mice. 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 (NTP 1992a). However, lifetime inhalation exposures of rodents to ethylbenzene did produce pathological lesions in the mouse liver (eosinophilic foci, hepatocyte syncytial alteration, hypertrophy, and necrosis) and rat kidney (renal tubular hyperplasia and chronic progressive nephropathy [CPN]), so the toxicological significance of the early subchronic changes in these organs cannot 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 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, lifetime exposure to ethylbenzene of 750 ppm produced lung pathology (alveolar epithelial metaplasia) and thyroid pathology (thyroid follicular cell hyperplasia), and ≥ 250 ppm of ethylbenzene produced hyperplasia of the pituitary gland—pars distalis. Rats that received lifetime 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 (NTP 1999).

As with inhalation exposure, liver and kidney effects were observed in subchronic oral studies conducted in rats at dosages of ≥ 250 mg/kg bwt ethylbenzene (Mellert et al. 2004, 2007, Li et al. 2010). 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, although the use of different strains across studies could account for some of the variation in response. One subchronic oral study also detected a minimal regenerative anemia and a reduction in prothrombin time, both of questionable significance (Mellert et al. 2004, 2007).

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 bwt, and increases in organ weights, ethylbenzene was fetotoxic causing decreases in fetal bwts and increases in skeletal variations (Andrew et al. 1981, Hardin et al. 1981, Saillenfait et al. 2003, 2006). 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 (Faber et al. 2006). 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 of ethylbenzene. Also, no adverse effects were seen on ovarian follicle counts, the pup litter parameters of pup sex ratios, live litter sizes, number of dead pups, viability indices, pup bwts, 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 exposure to 500 ppm of ethylbenzene (Faber et al. 2006). In the pilot study to the reproductive toxicity study (Stump 2004a), dose-related decreases in offspring pre-weaning and post-weaning bwts, as well as offspring survival immediately following weaning and initiation of exposure occurred at ≥ 500 ppm of ethylbenzene; however, these effects were not reproduced at 500 ppm in the definitive reproductive toxicity study (Faber et al. 2006).

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 of ethylbenzene vapor administered for 28 days (Stump 2004b, Li et al. 2010). 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 (NTP 1992a, 1999, Mellert et al. 2004, 2007).

Metabolism and pharmacokinetics

The disposition of ethylbenzene in animals and humans has been well characterized (Figure 4). 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. The metabolic pathways for ethylbenzene have been characterized and are presented in more detail in the “Cancer mode of action” section below. Physiologically based pharmacokinetic (PBPK) models developed to describe ethylbenzene disposition in rats, mice, and humans are discussed in the Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157.

Carcinogenicity

Ethylbenzene is tumorigenic in animals following lifetime exposures to high vapor concentrations (NTP 1999) (Table 3). NTP concluded that there was clear evidence of carcinogenicity in male rats based on the increased incidence of kidney tumors at 750 ppm of ethylbenzene. This exposure concentration falls above the point at which ethylbenzene metabolism becomes saturated, and also significantly reduced male rat survival. NTP concluded that there was some evidence of kidney tumors in female rats at this concentration; however, these tumors were detected only after extended histopathological evaluation. Male rats that inhaled 750 ppm of ethylbenzene
Table 3. Summary of key neoplastic and non-neoplastic effects of ethylbenzene in rats and mice (NTP 1999, Chan et al. 1998).

| Species | Sex | Concentration (ppm) | Chronic progressive nephropathy (mean severity score) | Adenoma/ carcinoma* | Kidney | Testes | Lung | Liver |
|---------|-----|---------------------|------------------------------------------------------|---------------------|--------|--------|------|-------|
| Rat     | M   | 0                   | 47/50 (2.3)                                          | 3/50                | 14/50  | 36/50  | 2/50 | 5/50  |
|         |     | 75                  | 43/50 (2.4)                                          | 5/50                | 19/50  | 33/50  | 2/50 | 1/50  |
|         |     | 250                 | 47/50 (2.3)                                          | 8/50                | 12/50  | 40/50  | 1/50 | 0/50  |
|         | F   | 75                  | 48/50 (3.5)                                          | 21/50               | 8/50   | 44/50  | 2/50 | 0/50  |
|         |     | 75                  | 42/50 (1.6)                                          | 0/50                | NA     | 1/50   | 5/50 | 1/50  |
|         |     | 250                 | 43/50 (1.7)                                          | 1/50                | 2/50   | 1/50   | 5/49 | 0/49  |
|         |     | 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  |
|         |     | 75                  | 38/50                                               | 0/50                | 1/50   | 0/50   | 6/50 | 10/50 |
|         |     | 250                 | 40/50                                               | 1/50                | 0/50   | 1/50   | 4/50 | 15/50 |
|         | F   | 0                   | 36/50                                               | 0/50                | 0/50   | 1/50   | 10/50| 19/50 |
|         |     | 75                  | 13/50                                               | 0/50                | NA     | 0/50   | 4/50 | 5/50  |
|         |     | 250                 | 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.

also appeared to have an exacerbation in testicular or Leydig cell tumors (LCTs), a type of tumor that occurs in nearly all aged rats of this strain. NTP concluded that there was some evidence of liver and lung tumors in mice at 750 ppm of ethylbenzene. 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 (NTP 1999). Increases in regenerative cell proliferation are postulated to play a key role in the mouse tumor findings (Stott et al. 2003).

**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 (Barnett 2006, Faber et al. 2007, Li et al. 2010). Specialized investigations of ethylbenzene effects on hearing do indicate that ethylbenzene can cause ototoxicity. Otitotoxicity has been reported for other aromatic hydrocarbons and a 13-week study in rats found alterations in brainstem auditory evoked responses and outer hair cell (OHC) morphology in rats at concentrations of 200 ppm and greater ethylbenzene (Gagnaire et al. 2007). Therefore, hearing effects may be a concern for ethylbenzene.

**Developmental neurotoxicity**

No available evidence indicates that ethylbenzene is 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 (Faber et al. 2007). 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 of ethylbenzene. It should be noted, however, that this study did not specifically address ototoxicity, the most sensitive indicator of neurotoxicity in adult rats (Gagnaire et al. 2007), in detail (e.g., no cochlear histopathology was reported). Nonetheless, the negative findings for acoustic startle response were supportive of a lack of selective ototoxic affect.

**Human data**

Acute lethality or serious poisoning in humans has 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 Bardodejová 1970). Dizziness was reported in human subjects with 6-min 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 Bardodejová 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 and Thomas, 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 et al. 1975).

There are no reliable human studies or reports on subchronic, chronic, reproductive and developmental, 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 and gasoline) that contained ethylbenzene as a component; however, these studies cannot be used to reliably assess ethylbenzene toxicity in a manner suitable for toxicity reference value derivation and quantitative risk assessment due to confounding exposures and small study sizes. Although not specifically informative on quantitative assessment of ethylbenzene toxicity, a few of the most commonly referenced human studies are briefly described below.

Angerer and Wulf (1985) studied 35 sprayers working with alkyd, phenol, and polyester varnishes dissolved mainly in mixed xylene 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 their neurobehavioral effects. The study consisted of 105 house painters and 53 control/non-painter workers. The concentration of workplace ethylbenzene was found to be up to 12.9 mg/m³ (3 ppm) and there was also 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 xylens, 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 of the MAK values. Other chemicals that were present (e.g., resins and 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 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 0.43–17.2 mg/m³ (0.1–4 ppm) for 4–20 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 (Bardodĕj and Čírek 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 0.2–0.3 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, leukocytes, 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 2–24 years to hydrocarbons. The hydrocarbon concentrations determined at the factory were 220.7–234 mg/m³ (50–54 ppm) ethylbenzene, 31.9–47.8 mg/m³ benzene, 189.7–212.5 mg/m³ toluene, and 47–56.4 mg/m³ xylene. The rubber factory workers were compared with 42 unexposed administrative office workers. The results of this study found that the exposed group had fewer semen with normal characteristics (17%) compared with 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 two-generation rat reproduction study that did not find any abnormal sperm or fertility effects following exposure to high concentrations of ethylbenzene (Stump 2004a, Faber et al. 2006).

Only one study that discussed genotoxic effects in humans after inhalation exposure to a mixture of chemicals, including ethylbenzene, was found. Holz et al. (1995) determined 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 2340 mg/m³ (84–539 ppm). Measurements performed at the pump house showed ethylbenzene concentration levels > 4000 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 PI-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-esophageal dysmotility-sclerodactyly-telangiectasia syndrome of scleroderma or CREST patients. Metabolite concentrations in urine of exposed workers confirmed absorption of the ethylbenzene. No genotoxic effects related to exposure were detected by DNA adduct formation or DNA single-strand breaks and sister chromatid exchange. Increased 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 xylene. In addition, the sample size of 25 exposed workers and 25 non-exposed controls was very small.

No reliable human studies or reports on auditory toxic effects for ethylbenzene in the absence of noise are available. While there is some evidence of hearing effects in workers exposed to ethylbenzene and other aromatic hydrocarbons, co-exposure to noise complicates interpretation of the human studies. A recent study by Zhang et al. (2013) reported increased odds ratios for hearing loss (25dB or more) in petrochemical workers (petrochemical groups 1 [n = 246 males] and 2 [n = 307 males]) exposed to both ethylbenzene and noise as compared with a control group of office personnel in the plants (n = 327, gender not specified). An additional group exposed to noise but not petrochemicals (power station group [n = 290 males]) was also evaluated. When matched for age, cigarette smoking, and alcohol drinking, the hearing loss odds ratio (95% confidence interval or CI) was 86.4 (28.4–452) for petrochemical group 1, 124 (11.7–651) for petrochemical group 2, and 15.3 (5.7–52.9) for the power station group. The petrochemical groups (1 and 2) and power station group had similar levels of noise (82.7, 83.5, and 84.3 dB(A), respectively), which were higher than the noise levels in the offices (67.3 dB(A)); no information on variability of noise levels within each work environment was provided. The reported ethylbenzene levels were 123 ± 23 mg/m³ and 135 ± 32 mg/m³ (approximately 28 and 31 ppm) for petrochemical groups 1 and 2 and not detectable (below the limit of detection of 1.3 mg/m³) for the control group. Styrene, benzene, toluene, and xylene levels were below 1.7, 0.6, 1.2, and 3.3 mg/m³ for all workplaces. The description of the air sampling was minimal; no details were provided with respect to the type of samples (area vs. personal) or the timing of the samples relative to the response testing or the work histories of the tested individuals (recent vs. historical). While the petrochemical groups (ethylbenzene + noise) had higher hearing loss odds ratios than the control group and the power plant group (noise only), the odds ratios for the petrochemical groups overlap with those of the power plant group; no direct comparison of the petrochemical groups to the power plant group was made. Thus, while the study suggests a role for ethylbenzene in potentiating hearing loss in noise-exposed workers, the effect of ethylbenzene alone on hearing loss cannot be determined from this study.

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 Círek 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 were provided to substantiate the author’s assertions, there was co-exposure 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.

**Toxicity reference value derivation**

Ethylbenzene has been extensively tested for toxicity (see “Hazard assessment” section). An evaluation of the cancer endpoints, potential MOAs, and cancer potency was considered separately from the non-cancer endpoints (see “Cancer dose–response assessment for ethylbenzene” section). Existing non-cancer reference concentration (RfC) and reference dose (RfD) values from the IRIS were derived in 1991 and 1988, respectively (US EPA 1991). Since that time, many additional studies pertaining to the toxicity, toxicokinetics, and potential MOA of ethylbenzene toxicity have been conducted; this newer material was incorporated into the non-cancer and cancer toxicity derivations in the subsequent sections. Proposed reference values that reflect the current state of knowledge regarding the non-cancer and cancer effects include the following:

- An inhalation RfC of 0.3 ppm was derived based on ototoxicity observed in rats exposed to ethylbenzene.
- An oral RfD of 0.5 mg/kg bwt/day was derived based on liver effects observed in mice exposed to ethylbenzene.
- A cancer reference value of 0.48 ppm (daily ingestion rate of 0.71 mg/kg bwt/day) was derived based on lung tumors in mice exposed to ethylbenzene.

The methods used to derive these toxicity values and the results are described below.
Non-cancer toxicity reference values (RfC and RfD) for ethylbenzene

Outline of key decisions for non-cancer reference value derivation

Reference values for ethylbenzene were derived using the following equation:

\[
\text{RfV} = \frac{\text{BMD}}{\text{UFA} \times \text{UFH} \times \text{UFS} \times \text{UFL} \times \text{UFD}}
\]

Where

\( \text{RfV} \) = Reference value.

\( \text{BMD} \) = Benchmark dose.

\( \text{UFL} \) = Uncertainty factor for effect level extrapolation. Uncertainty results when an “effect” level (lowest observed adverse effect level [LOAEL]) rather than a “no effect” level (no observed adverse 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.

\( \text{UFS} \) = 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.

\( \text{UFA} \) = 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).

\( \text{UFH} \) = 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.

\( \text{UFD} \) = Uncertainty factor for database sufficiency. The lack of an extensive testing database (e.g., two-generation reproductive toxicity, chronic studies, and 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. The proposed MOAs for endpoints and their relevance to humans were evaluated and their corresponding internal dose metrics were selected. MOAs were considered relevant to humans unless otherwise specified below. Candidate studies/effects were considered suitable for RfC/RfD derivation if the reporting of the study was adequate (e.g., full papers published in English, with detailed methodological information, rather than limited abstracts) and investigated relevant toxicological endpoints (e.g., apical toxicological endpoints or their precursors). 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. The calculated internal doses were used for dose–response analysis using US 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., lower confidence limit on dose producing an effect of 10% [LED10]) was identified.

3. Uncertainty factors were applied to the internal dose point of departure, and the human PBPK model was used for interspecies extrapolation (and dose route extrapolation, as needed) to derive external dose 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.

The National Research Council (NRC 2014) has recommended that US EPA consider alternative practices for toxicity reference value derivation that include Bayesian hierarchical methods of uncertainty analysis. This proposed method replaces traditional point of departure (BMD or NOAEL) and uncertainty factor approach as shown above with a central tendency estimate and lower bound (e.g., two-sided 95% lower confidence limit). The proposed procedure incorporates multiple levels in a hierarchy based on relevance to human risk, with potential dose–response descriptors (e.g., BMDs) within a level combined on the basis of exchangeability (i.e., “equally likely to reflect human cancer [or non-cancer] risk”). Precisely how such an approach would be implemented for multiple study designs, different endpoints, and considering multiple routes of exposure, as is the case with the ethylbenzene database, extends far beyond the example provided by NRC (2014). Lacking sufficient, concrete guidance on how to implement such an analysis, the traditional approach was used for this assessment.

Key studies

Several studies were considered as a potential basis for the RfC and RfD. These select studies are summarized briefly in Table 4.

The only significant non-cancer findings of the NTP (1999) chronic rat toxicity study were related to CPN, a rat disease with no human correlate (Hard 2002), so none of the non-cancer effects observed in rats in this study are likely to be relevant to human risk (see discussion below, “Proposed MOA for kidney tumors” section). This study can thus be interpreted...
as supporting a NOAEL of 750 ppm for non-cancer effects relevant to human health. Since 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. Likewise, the inhalation adult general neurotoxicity study (Li et al. 2010), the two-generation study (Faber et al. 2006), and developmental toxicity studies of Andrew et al. (1981), Hardin et al. (1981), Saillenfait et al. (2003), and Faber et al. (2007) also have sufficiently high NOAELs that toxicity reference values were not derived. Furthermore, ethylbenzene was not a selective developmental toxicant, so the derived NOAELs and LOAELs from reproductive and developmental toxicity studies correspond to maternally toxic levels for effects that are more appropriately characterized in longer (subchronic or chronic) studies. RfC and RfD derivation based on the findings of ototoxicity in the rat (Gagnaire et al. 2007), liver and pituitary effects in the mouse (NTP 1999), liver and blood effects by the oral route in a subchronic rat study (Mellert et al. 2004, 2007), and liver effects in a second subchronic rat study (Li et al. 2010) are presented below.

### Table 4. Studies considered for ethylbenzene RfC/RfD derivation.

| Endpoint                              | Species | Reference          | NOAEL               | LOAEL               |
|---------------------------------------|---------|--------------------|---------------------|---------------------|
| Kidney (incidence), mortality         | Rat     | NTP (1999)         | 250 ppm             | 750 ppm             |
| Kidney (severity)                     | Rat     | NTP (1999)         | Not determined      | Not applicable      |
| Fertility and reproduction            | Rabbit  | Andrew et al. (1981), Hardin et al. (1981) | 500 ppm (EPA), 1000 ppm (American Chemistry Council 2007) | 1000 ppm (EPA), Not applicable (American Chemistry Council 2007) |
| Developmental toxicity                | Rat     | Andrew et al. (1981), Hardin et al. (1981) | 500 ppm             | 1000 ppm            |
| Developmental toxicity                | Rat     | Saillenfait et al. (2003) | 500 ppm             | 1000 ppm            |
| Ototoxicity                           | Rat     | Gagnaire et al. (2007) | Not determined (OHC loss) | 200 ppm (OHC loss, LOEL) |
| Liver, pituitary                      | Mouse   | NTP (1999)         | 75 ppm              | 250 ppm             |
| Liver and blood effects               | Rat     | Mellert et al. (2004, 2007) | 75 mg/kg bwt/day    | 250 mg/kg bwt/day   |

### 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 (RPT). The ototoxic effects of ethylbenzene are likely related to the irreversible loss of OHCs 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). However, central auditory effects of hydrocarbons have been reported for xylene-exposed laboratory workers (Fuente et al. 2013) and for jet fuel-exposed rats in the absence of cochlear/peripheral nervous system (Guthrie et al. 2014). No ototoxic effects were observed in Wistar rats consuming 5000 mg/L of 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 the metabolites are not responsible for toluene-induced ototoxicity (Pryor et al. 1991).

The proposed internal dose metric is the area under the concentration versus time curve (AUC) of ethylbenzene in RPTs (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% upper confidence limit (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 ± 4.24%, while OHC3 losses were 67.12 ± 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 (BMD) modeling.
- A UFS 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 with shorter exposures (1 month or less) (Johnson and Nylén 1995). A UFS of 1 was previously proposed for OHC3 losses (ACC 2006). As noted in the VCEEP meeting report (TERA 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 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 PBPK models appropriate for the interspecies extrapolation of tissue levels of ethylbenzene from rats to humans are available (see Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157), 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 US EPA RfC guidelines (US EPA 1994).
- A UFH of 10 as the default value is proposed. Sensitivity analyses for AUCR predictions in humans indicate that this value of UFH is adequate for protection of children (see Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157).
- 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 (1 × 3 × 3 × 10 × 1) is recommended for a potential ethylbenzene RfC based on rat ototoxicity (OHC3 loss).

NTP (1999), Mouse chronic toxicity study

Key findings

The NTP (1999) chronic cancer bioassay in mice also identified non-cancer 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 of ethylbenzene 6 h/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

The MOA for liver syncytial alteration in male mice is likely similar to the liver cancer MOA (discussed in “Cancer toxicity reference value derivation” section) mediated by the parent chemical. In mice, ethylbenzene produces multiple effects in the liver including enzyme induction, hepatocellular hypertrophy, mitotic figures, S-phase synthesis, and increased liver weight (Stott et al. 2003). This pattern is similar to that observed for phenobarbital (increased liver weight, hepatocellular hypertrophy, and mitotic figures) via activating the constitutive androstane receptor (CAR) (Geter et al. 2014). 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 non-cancer 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 (AUCL). 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.

The MOA for pituitary hyperplasia in female mice is potentially related to dopamine depletion by ethylbenzene metabolites. Dopamine secreted by the arcuate nucleus and periventricular nucleus of a rodent hypothalamus inhibits the secretion of prolactin from the anterior pituitary gland either by inhibiting lactotroph proliferation in the mouse pituitary gland (Saiardi et al. 1997) or by inhibiting the hypothalamic paraventricular nucleus that secretes prolactin-releasing hormone (see review by Ben-Jonathan and Hnasko 2001). Dopamine depletion in brain has been observed in rabbits exposed systemically to ethylbenzene or its metabolites mandelic and phenylglyoxylic acid (Mutti et al. 1988). Although there is no study documenting the mechanism of ethylbenzene-mediated dopamine depletion, a plausible mechanism is through inhibition of dopamine uptake into synaptic vesicles since styrene, a chemical similar to ethylbenzene, has been shown to block specific uptake of dopamine into synaptic vesicles prepared from rat brain striate in a dose-dependent manner, functioning as an indirect dopamine antagonist (Chakarabarti 1999). Sexual dimorphism in pituitary prolactin levels, providing an additional proliferative signal, could 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/bwt. 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 of parent compound would increase more dramatically.

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, 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 Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157), 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 US EPA RfC guidelines (US 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 protection for children (see Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157).
- An uncertainty factor for database sufficiency (UFD) = 1 is proposed because ethylbenzene has been extensively tested by the inhalation route.

Therefore, a composite UF of 30 (1 × 1 × 3 × 10 × 1) is recommended for a potential ethylbenzene RfC based on this study.

Study and endpoint-specific RfC derivations

RfC for ototoxicity

The only acceptable fit obtained for the selected dose metric was found for the Hill model (Akaike’s Information Criterion [AIC] = 160, \( p = 0.642 \)) (Table 5). The saturation of the response indicated by the Hill model is consistent with the data (Figure 1), which showed similar increases in OHC3 loss at 600 and 800 ppm (85.6 ± 7.7 and 90.8 ± 7.4%, respectively) (Gagnaire et al. 2007). The 95% UCL on the dose producing hair loss exceeding those in control rats (1.05%), LED0105, was 272.8 mg-h ethylbenzene/L of RPT/week, which is equivalent to an average tissue concentration of 1.6 mg/L.

Dividing the LED0105 (272.8 mg-h ethylbenzene/L of RPT/week) by the composite UF of 100 for this endpoint yielded a target human internal dose (AUCR) of 2.73 mg-h ethylbenzene/L of RPT/week. Weekly AUCR was calculated as the difference between the AUCR for 504 h (3 weeks) and AUCR for 336 h (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

The selected benchmark response was the 95% lower confidence limit on the dose producing a 10% increase in liver effects above the background incidence (LED10). A 10% increase in extra risk in considered to be the default benchmark response rate (US EPA 2005b), and is considered appropriate for this data set.

Using AUCL as the dose metric introduces considerable non-linearity 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. Since it is expected that PBPK modeling should resolve non-linearity 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 (data not shown).

Using the amount metabolized in the liver (normalized to liver volume, AM/ VL) as the dose metric, the best fit was provided by the Gamma, Q-Linear, and Weibull models (AIC = 151, \( p = 0.2801 \)), producing an LED10 = 3901 mg ethylbenzene metabolized/kg liver/week, (Table 6, Figure 2).

Dividing the LED10 (3901 mg ethylbenzene metabolized/kg liver/week) by the composite UF of 30 for this endpoint yielded a target human internal dose (AM/ VL) of 130 mg

| Model      | AIC | p value | ED0105 (mg/L-h/week) | LED0105 (mg/L-h/week) |
|------------|-----|---------|----------------------|-----------------------|
| Hill       | 160 | 0.642   | 319.6                | 272.8                 |
| Power      | 213 | <0.0001 | 24.1                 | 19.2                  |
| Polynomial | 215 | <0.0001 | 24.1                 | 19.2                  |
| Linear     | 222 | <0.0001 | 36.3                 | 29.4                  |

Table 5. BMDS output for Gagnaire et al. (2007) (loss of OHCs, row 3 in the cochlea).
ethylbenzene metabolized/kg liver/week. Weekly AM/VL was calculated as the difference between the AM/VL for 504 h (3 weeks) and AM/VL for 336 h (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**

The RfC for pituitary hyperplasia was 5.1 ppm, which rounds to 5 ppm. Details of the derivation of the RfC for this endpoint may be found in ACC (2007).

**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, an RfC of 0.3 ppm based upon ototoxicity was adopted for use in the ethylbenzene VCCEP risk characterization.

**Non-cancer RfD derivation**

Four studies were considered as potential bases for the non-cancer RfD, a 90-day repeated gavage study for general toxicology in rats (Mellert et al. 2004, 2007), a 90-day adult general neurotoxicity study by gavage in rats (Li et al. 2010), 13-week inhalation ototoxicity study in rats (Gaignaire et al. 2007), and chronic inhalation toxicology study in mice (NTP 1999).

**Mellert et al. (2004, 2007) and Li et al. (2010) oral subchronic rat studies**

**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 h 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 of 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). Increased liver weights were also observed at 250 and 500 mg/kg bwt/day (NOAEL, 50 mg/kg bwt/day) in another subchronic study where ethylbenzene was administered to rats twice daily by gavage (Li et al. 2010).

**Proposed MOA and internal dose metrics**

The MOA for liver effects in male and female rats is likely similar to the mouse liver cancer and non-cancer MOA (discussed above). Liver effects were not observed in male and female rats following lifetime exposure to 1-phenylethanol (NTP 1990), a metabolite of ethylbenzene, suggesting this endpoint is best attributed to the parent chemical. Studies conducted in pregnane X receptor or PXR- and CAR-humanized mice suggest that human receptors are able to support PB-induced hypertrophic responses, but not the hyperplastic responses in the liver (Ross et al. 2010). For this reason, the non-cancer effects of ethylbenzene on rat liver are assumed to be relevant to human health.

The MOA 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 RPTs lacking any known or anticipated capability for ethylbenzene metabolism, the most relevant dose metrics are AUC in RPTs and amount metabolized normalized to bwt.

**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. The impact of an alternative UFS of 3 was considered for
the liver effects observed in Mellert et al. (2004, 2007), as discussed in the following section; a smaller UFS for these effects is supported by the similar incidence and severity (as determined by histopathology grades) of centrilobular effects resulting from 4 weeks versus 13 weeks of treatment.

- A UFA of 3 is proposed. As noted above, 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 Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157), 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. Since 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 protection for children (see Supplementary Material to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157).

- 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, 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 (1 × 10 × 3 × 10 × 1) is recommended for the ethylbenzene RfD.

**RfD derivations from oral studies**

*RfD derivation for liver effects observed in oral studies*  
Mellert et al. (2004, 2007). Based on the similarity of the responses in males and females, the data sets were combined. Since it is expected that PBPK modeling should resolve non-linearity 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 (Table 7). The best fit for AM/VL was generated using the quantal-quadratic model (AIC = 61.4, p = 0.576) (Figure 3), resulting in an LED10 of 1546 mg ethylbenzene metabolized/kg liver/day.

Dividing the LED10 (1546 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 h (15 days) and AM/VL for 336 h (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. If UFS was reduced from 10 to 3, the candidate RfD for this study and endpoint would be 0.45 mg/kg bwt/day, which rounds to 0.5 mg/kg bwt/day.

Li et al. (2010). As with the Mellert et al. (2004, 2007) study, the responses in males and females were similar, so the data sets were combined. Based on the findings from analysis of the Mellert et al. (2007) data, only AM/VL was considered as a dose metric. Upon inspection of the data in the original report (Barnett 2006), apparent transcription errors in the preparation of the report by Li et al. (2010) were identified. The relative liver weights (sample number) for male rats used for BMD analysis were 3.68±0.35% (10), 3.78±0.45% (10), 4.19±0.35% (9), and 4.73±0.51% (9) for the control, 50, 250, and 500 mg/kg/day groups, respectively. For female rats, the relative liver weights (sample number) were 3.77±0.34% (9), 3.82±0.36% (9), and 4.13±0.49% for the control, 50, and 250 mg/kg/day groups, respectively. The BMD analysis determined BMDLs for benchmark responses of an increase in liver weight equivalent to 1 standard deviation (BMDL1SD) or 10% increase in relative liver weight.
(BMDLR10). The best fit was generated using the Hill model ($p = 0.433$). The BMDL1SD and BMDLR10 were 3073 and 2946 mg ethylbenzene metabolized/kg liver weight/day, respectively. As these potential points of departure were higher than those derived for liver effects in the Mellert et al. (2004, 2007) study and thus would result in higher candidate RfDs, this endpoint was not carried forward in the assessment.

**RfD derivation for regenerative anemia and prothrombin time**

The RfDs derived for these endpoints, 0.4 mg/kg bwt/day for regenerative anemia and 0.6 mg/kg bwt/day for prothrombin time, were both larger than for liver effects in the same study, and thus not considered appropriate candidates for the chronic non-cancer RfD. Their derivation (with UFS of 10) was presented in greater detail in ACC (2007).

**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.

**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. Further, if a smaller UFS value of 3 (rather than the default value of 10) were used in deriving the RfD for liver effects based on the Mellert study, the two liver-based RfDs would be equal. 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 non-cancer reference value for oral exposure to ethylbenzene.

**Cancer dose–response assessment for ethylbenzene**

Currently, ethylbenzene is considered not classifiable as to human carcinogenicity (Group D) by US EPA (1991). However, since the time of US 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 two-year 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 US EPA’s framework described in its *Guidelines for Carcinogen Risk Assessment* (US EPA 2005b).

**Methods**

The published literature was reviewed regarding the toxicity, carcinogenicity, toxicokinetics, and MOA for ethylbenzene. This information was used to prepare the following sections:

- **Cancer Hazard Identification Summary:** This section provides a summary of the cancer hazard identification described in greater detail in ACC (2007).
- **Mode of Action:** This section provides an assessment of possible 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 MOA (Meek et al. 2003, US EPA 2005b, Boobis et al. 2006, 2009; Meek et al. 2014) were considered in this analysis.
- **Dose–Response Assessment:** 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 Supplementary Material, Supplement A to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157). 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 MOA.
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 processes were 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 processes were performed using US EPA’s 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 non-linearity in the dose–response relationship.
6. **Cancer Value Presentation**—Consistent with US EPA guidelines (US EPA 2005b), cancer potency estimates
included characterization of central tendency, upper bound, and lower bound values.

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 (Bardoděj and Círek 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, and toluene), 17 cancer deaths were reported versus 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 NTP cancer bioassay, groups of 50 male and 50 female F344/N rats and B6C3F1 mice were exposed to 0, 75, 250, or 750 ppm of ethylbenzene via inhalation for 6 h/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 in 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) testicular tumors (LCTs) in male rats; (3) alveolar/bronchiolar adenoma and carcinoma (combined) in male mice; and (4) hepatocellular adenoma and carcinoma (combined) in female mice (Table 3). Significant non-neoplastic effects were also observed in target tissues including 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 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. With respect to precursor lesions, the incidence and severity of nephropathy, as well as the incidence of renal tubular cell hyperplasia, were increased in male rats (NTP 1990).

Groups of 50 male and 50 female Sprague-Dawley rats were exposed to 0 or 800 mg/kg-day of ethylbenzene via oil gavage for 4 days/week up to 104 weeks, with the 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 of ethylbenzene via oil gavage for 4 days/week up to 145 weeks. Survival was affected in all the 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; however, these were not observed in either sex exposed to 500 mg/kg-day. An increase in total malignant tumors was observed in both sexes, but the authors concluded that the increase was non-dose-related.

Cancer mode of action

An evaluation of the MOA by which ethylbenzene produces tumors in rodents was conducted with consideration of frameworks created by US EPA and ILSI RSI/IPCS (Meek et al., 2003, 2014; US EPA 2005b, Boobis et al. 2006, 2009). 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.

Question 3 above addresses whether the human relevance of animal tumors can be excluded on the basis of a potential quantitative difference in kinetic behavior between animals and humans. Such a consideration is particularly important in the case of ethylbenzene-induced tumorigenicity. In recent years, several reviews have emphasized that rodent carcinogenicity responses restricted to high test doses may have questionable human health hazard and risk relevance. Thus, high-dose specific saturation of metabolic processes, as evidenced by appearance of non-linear toxicokinetics, may result in transition to novel modes of carcinogenic action unique to those high-dose levels and which are differentiated from potential alternative MOAs operating at lower non-saturating animal doses and substantially lower real-world human exposures (Foran 1997, Conolly et al. 1999, Slikker et al. 2004a,b, Barton et al. 2006, Carmichael et al. 2006, Doe et al. 2006).

The lack of human relevance of high-dose toxicity findings observed only under conditions of doses that exhibit saturation of metabolic processes resulting in non-linear toxicokinetics has been recognized in OECD guidance for dose selection in animal bioassays (OECD 2011). In dose selection guidance offered for the Extended One-generation Reproduction Test, OECD recommended the following regarding appropriate dose selection:

“If TK data are available which indicate dose-dependent saturation of TK processes, care should be taken to avoid high dose levels which clearly exhibit saturation, provided of course, that human exposures are expected to be well below the point of saturation. In such cases, the highest dose level should be at, or just slightly above the inflection point for transition to non-linear TK behaviour.”

Although the above guidance was offered for a reproduction study, the recommendations also are appropriate for dose selection in other animal bioassays including carcinogenicity studies (OECD 2009), and particularly so for substances that are not regarded as genotoxic as is the case for ethylbenzene.

Recently, the concept of using toxicokinetic data to guide
dose selection in chronic bioassays, as a substitute for the traditional Maximum Tolerated Dose or MTD approach, has been termed the Kinetically derived Maximum Dose or KMD (Saghir et al., 2012).

There is ample evidence that ethylbenzene-induced tumors, which were restricted to only the highest inhalation dose tested (NTP 1999, Table 3), occurred under conditions of high-dose-specific saturation of metabolism of ethylbenzene as evidenced by onset on non-linear toxicokinetics at inhalation doses well below the top tested dose of 750 ppm. Charest-Tardif et al. (2006) demonstrated that 4-h exposures of mice to 75, 200, 500, and 1000 ppm of ethylbenzene, representing 2.5-, 6.7-, and 13.3-fold increased increments in exposure relative to the lowest 75 ppm tested exposure, resulted in dose-disproportionate 4.3-, 36.2-, and 155.4-fold increases in blood C_{\text{max}} concentrations in the 200, 500, and 1000 ppm treatments. The onset of non-linear toxicokinetics in mice at exposures as low as 200 ppm was further evidenced by 4.7-, 40.8-, and 215.9-fold dose-disproportionate increases in blood ethylbenzene AUC values (relative to 75 ppm). Evidence of onset of non-linear toxicokinetic behavior also was apparent in rats exposed to approximately 100–200 ppm of ethylbenzene. Tardif et al. (1997) reported a relative 3.75-fold increase (graphically estimated) in blood C_{\text{max}} concentrations compared with a 2-fold increase in ethylbenzene exposures (100 and 200 ppm, 4 h). Using the PBPK model developed by Tardif et al. (1997), Krishnan (unpublished; ACC 2007) calculated blood C_{\text{max}} concentrations of 0.63, 3.88, and 46.1 μg/ml and blood AUC values of 15.6, 88.3, and 1265.1 following single 4-h exposures to 25, 100, and 500 ppm of ethylbenzene. The 100 and 500 ppm values both represented dose-disproportionate increases of 6.2- and 73.2-fold and 5.7- and 81.2-fold for blood C_{\text{max}} AUC values, respectively.

The robust evidence of non-linear toxicokinetics in mice and rats indicates that, if current OECD guidance regarding dose selection had been considered in the ethylbenzene chronic cancer bioassay, a top exposure of approximately 250 ppm would have been justified, that is, “...at, or just slightly above the inflection point for transition to nonlinear TK behaviour.” Importantly, such a 250-ppm top exposure would have resulted in a toxicologically valid bioassay design that would not have identified any ethylbenzene-induced tumors. Extensive information regarding human ethylbenzene environmental exposures (see “Exposure assessment” section) demonstrates that ethylbenzene also fulfills the additional OECD cautionary guidance that states, in order to use toxicokinetic data to inform top dose selection in animal bioassays, “…human exposures are expected to be well below the point of saturation” (OECD 2011). The 250 ppm (870,000 μg/m³) mid-dose used in the NTP (1999) bioassay is substantially above environmental ethylbenzene concentrations in both outdoor and indoor air (see Concentration of ethylbenzene in potential exposure media). Although this cancer MOA section evaluates the available data characterizing the potential human relevance of MOAs of the various tumor responses associated only with the exposure to 750 ppm of ethylbenzene, it must be kept in mind that these tumor responses are likely lacking human relevance based on demonstration of distinct quantitative differences in kinetic factors impacting doses producing tumors in animals (high-dose specific and above inflection point of onset of non-linear toxicokinetics) as compared with ethylbenzene kinetics projected in humans under conditions of real-world human exposures (Question 3 above). The availability of high-quality toxicokinetic and human exposure data thus indicates that a lack of complete batteries of biological MOA information addressing each of the high-dose-specific ethylbenzene rodent tumors, as is the case for some of the tumor endpoints addressed below, is not a critical deficiency to otherwise excluding the human relevance of such tumor findings.

The MOAs by which each of the rodent tumor types identified in the Cancer Hazard Identification Summary (rat kidney, mouse liver, mouse lung, and rat testes) are summarized in Table 8 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. Since the proposed MOAs for ethylbenzene involve the formation of metabolites, the metabolic pathways for side-chain oxidation (Figure 4, Box A) and ring oxidation (Figure 4, Box B) are provided (Saghir et al. 2009).

### 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

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**Table 8. Comparison of possible MOAs for ethylbenzene-induced tumors.**

| Group              | Criteria                        | Proposed MOAs for specific tumors | Default MOA for all tumors |
|--------------------|---------------------------------|----------------------------------|---------------------------|
| Hill criteria      |                                 | Rat kidney (Non-genotoxic) Mouse liver (Non-genotoxic) Mouse lung (Non-genotoxic) Rat testes (Non-genotoxic) | (Direct genotoxicity) |
| Strength of association | +                              | +                                | +/−                       | **in vivo** +/− |
| Consistency of association | +                              | +                                | +/−                       | **in vitro** +/− |
| Specificity of association | +                              | +                                | +/−                       | −               |
| Dose–response concordance | +                              | +                                | +/−                       | −               |
| Temporal relationship | +                              | +                                | +/−                       | −               |
| Coherence and plausibility | +                              | +                                | +/−                       | −               |
| 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.
following key events: (1) exposure to ethylbenzene; (2) absorption; (3) distribution of ethylbenzene to tissues; (4) metabolism of ethylbenzene in liver and lung to 1-phenylethanol; (5) distribution of 1-phenylethanol to kidney; (6) exacerbation of CPN; and (7) progression to cancer (Table 9). The weight of evidence for this MOA is considered 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 (NTP 1999). 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

Figure 4. Metabolism of ethylbenzene in relation to the potential MOAs for tumor formation (Saghir et al. 2009).

A: Primary Rat, Mouse (Liver), Human Metabolism (High-Dose, Threshold)
B: Enhanced Mouse (Clara Cell) Metabolism (Mouse-Specific)
Table 9. Key events in the MOA for ethylbenzene-induced rat kidney tumors.

| Event                          | Evidence in rodents                          | Evidence in humans                          | Potentially inconsistent evidence | Qualitative differences between rodents and humans | Quantitative differences between rodents and humans | Sources of non-linearity that may impact high-to-low dose extrapolation |
|--------------------------------|----------------------------------------------|---------------------------------------------|----------------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------------|
| 1. Exposure                    | Exposures to ethylbenzene 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 (see “Exposure assessment” section) | None identified. | Exposures to EB are isolated in animals, while human exposures to EB occur along with exposures to other chemicals | Rodent exposures under the conditions of the NTP (1999) bioassay (up to 750 ppm) are orders of magnitude higher than expected human exposure | None identified. Linearity is assumed. |
| 2. Absorption                  | EB is well absorbed following inhalation (Chin et al. 1980) or ingestion (El Mastri et al. 1956). Absorption by the skin is also rapid if evaporation is impeded (Tsunata 1982, Morgan et al. 1991, Susten et al. 1990). | EB is well absorbed following inhalation (Bardodej and Bardodejova 1970, Engström and Bjurström 1978, Aastrand et al. 1978, Gromiec and Piotrowski 1984) and dermal (Dutkiewicz and Tiras 1967) routes. EB is assumed to be well absorbed via ingestion. | None identified. | None identified. The processes dictating absorption are assumed to be qualitatively similar. | None identified. The processes dictating absorption are assumed to be quantitatively similar. | None identified. Linearity is assumed. |
| 3. Distribution of parent chemical to the liver | Absorbed EB is rapidly distributed to all tissues in the body (Chin et al. 1980, Engström et al. 1985, Cappuert 2000) | Rapid distribution of EB to human tissues is assumed. | None identified. | None identified. The processes dictating distribution are assumed to be qualitatively similar. | None identified. The processes dictating distribution are assumed to be quantitatively similar. | None identified. Linearity is assumed. |
| 4. Metabolism to active metabolite | Cytochrome P450-mediated reactions produce alky side chain and ring oxidation of EB (McMahon and Sullivan 1966, 1968, Engström 1984, Kaubisch et al. 1972, Stott et al. 2003, Saghir et al. 2006, 2009, Cossec et al. 2010) | Cytochrome P450-mediated reactions produce alky side chain and ring oxidation of EB (Engström et al. 1984, Sams et al. 2004, Saghir et al. 2006, 2009) | None identified. | 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 et al. 2006, 2009) | Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation) |
| 5. 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. | None identified. The processes dictating distribution are assumed to be qualitatively similar. | None identified. The processes dictating distribution are assumed to be quantitatively similar. | None identified. Linearity is assumed. |
| 6. Exacerbation of CPN         | EB produces a dose-dependent increase in the incidence and severity of CPN in male and female rats (see Table 3; NTP 1999). A role for CPN in rat kidney tumors has been proposed (Travlos et al. 2011, Hard 2002, Hard et al. 2012, 2013) | | No evidence. | | | |
| 7. Kidney tumor formation      | Increased incidence of kidney tumors in male and female rats (see Table 3; NTP 1999) | | No evidence. | | | |

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Potentially inconsistent evidence

Qualitative differences between rodents and humans

Quantitative differences between rodents and humans

Sources of non-linearity that may impact high-to-low dose extrapolation
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. Travlos et al. (2011) assessed data for 24 chemicals and reported an association between CPN exacerbation after 90 days of exposure and the subsequent formation of kidney tumors after 2 years of exposure. Similarly, Hard et al. (2012) examined kidney sections from 24 NTP cancer bioassays in male and female F344 rats. The authors reported a significant association between the advanced stages of CPN severity and the development of renal tubule tumors. This association was able to explain differences observed across sex (male > female) for the occurrence of renal tubule tumors. It has been shown that following ethylbenzene exposure, there is an accumulation of $\alpha_2\text{u}-\text{globulin}$ early after exposure, but deposition of $\alpha_2\text{u}-\text{globulin}$ 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 $\alpha_2\text{u}-\text{globulin}$ in accentuating CPN is suggested, however, in that additional subchronic studies provide evidence of hyaline droplets, a sign of $\alpha_2\text{u}-\text{globulin}$ accumulation, in proximal tubules (Stott et al. 2003, Mellert et al. 2004). $\alpha_2\text{u}-\text{Globulin}$ has previously been associated with renal tumors in the male rat. However, one of the histological criteria of $\alpha_2\text{u}-\text{globulin}$ tumors, granular casts and papillary mineralization, was 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 $\alpha_2\text{u}-\text{globulin}$-type MOA 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 (Engström 1984) and has been shown to be weakly carcinogenic with regard to the kidney and has increased the occurrence of CPN (NTP 1990). Based upon the rodent data for incidence and severity in Table 3, a strong correlation can be observed between the CPN and renal tumors, which appears to hold across species and sex (Figure 5). Irrespective 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% × 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 5). 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). This association appears to be consistent across chemicals (Travlos et al. 2011, Hard et al. 2012). 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 $\alpha_2\text{u}-\text{globulin}$ accentuation of the CPN MOA. 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% versus 0%, 0%, and 2% for controls and exposed animals, respectively (NTP 1999, Chan et al. 1998). Likewise, end-stage CPN was reported in 42% of male rats in the high-dose group and in 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). Using data for 24 chemicals, Hard et al. (2012) reported that the association between CPN and renal tumors was able to explain differences observed across sex (male > female) for the occurrence of renal tubule tumors. Additionally, in a review by Abrass (2000), a study performed by Baylis (1994) reported that castrated male rats were protected from CPN; however, ovariectomized 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 \( \alpha_{2u} \)-globulin is regulated by male sex steroids. Since 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 5), this MOA serves to explain the species- and sex-specific responses observed for kidney tumors in rodents 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 0–4 scale used by NTP in Table 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-Phenylenol, 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 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 of 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). Using data for 24 chemicals, Travlos et al. (2011) determined that the CPN exacerbation after 90 days of exposure was predictive of kidney tumor formation after 2 years of exposure. An increase in \( \alpha_{2u} \)-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-h exposure of ethylbenzene (up to 600 ppm) the major metabolite was identified as 1-phenylethanol (Engström 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, and is supported by observations made across chemicals (Travlos et al. 2011, Hard et al. 2012). However, CPN is a rodent-specific disease and considered irrelevant for extrapolating this MOA to humans (Hard 2002, Hard et al. 2009, 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 and cumene), in which an increased incidence of kidney tumors is 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 2009).

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 carcino-genicity bioassay, and CPN as the underlying prime influence, requires fulfillment of a set of specific criteria (Lock and Hard 2004):

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 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 with 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 should not be 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 9), critical qualitative differences between rats and humans exist. Of the major human renal diseases (renal vascular disease due to hypertension; diabetes; glomerulonephritis; and infective obstructive nephropathy), none have the singular features of CPN in the laboratory rat (Hard and Khan 2004, Hard et al. 2009). 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 (Rodríguez-Puyol, 1998). In the laboratory rat, CPN progresses 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 (Ruggenenti 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. Exacerbation by ethylbenzene of CPN, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as a process that does not occur in humans. US EPA’s Risk Assessment Forum advised risk assessors against using information on α2 u-globulin-mediated male rat renal tubule tumors to assess human health risks (Rogers and Baetcke 1993). 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 MOA 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, 2009); 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.

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 α2 u-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 of an alternative MOA (direct genotoxicity) is provided below (following proposed MOAs for other observed rodent tumors).

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 non-genotoxic MOA similar to phenobarbital (Elcombe et al. 2014). The key events in the MOA include (1) exposure; (2) absorption; (3) distribution of parent chemical to liver; (4) CAR activation; (5) altered gene expression; (6) increased cell proliferation; (7) clonal expansion of altered hepatic foci; and (8) liver tumor formation (Table 10). The weight of evidence supporting this MOA is evaluated using the modified Hill criteria below.
Table 10. Key events in the MOA for ethylbenzene-induced female mouse liver tumors.

| Event | Supporting evidence | Potentially inconsistent evidence | Qualitative differences between rodents and humans | Quantitative differences between rodents and humans | Sources of non-linearity that may impact high-to-low dose extrapolation |
|-------|---------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------------------------|
| 1-3. Exposure/Absorption/Distribution | Events are the same as described in Table 9 | | | | |
| 4. CAR activation | No evidence. Activation is assumed | None identified | None identified | None identified | None identified |
| 5. Altered gene expression | In rats, induction of CYP2B1 and CYP2B2 has been reported (Imaoka and Funae 1991, Sequeira et al. 1992, Yuan et al. 1997a,b). | Enzyme induction is assumed | None identified | Since 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 et al. 2006). | Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation) |
| 6. Increased cell proliferation | Increased liver weights are reported in mice (NTP 1992a; Cragg et al. 1989). Liver DNA synthesis and mitotic figures increased in mice (Stott et al. 2003) | No evidence | None identified | An increase in altered hepatic foci (eosinophilic) was observed only in female mice, but not in male mice or in rats of either sex (NTP 1999), and is not expected to occur in humans. | Phenobarbital exposure does not increase cell proliferation in human hepatocytes in vitro (Elcombe et al. 2014) |
| 7. Clonal expansion of altered foci | Eosinophilic foci are increased in female mice exposed to 750 ppm (see Table 3, NTP 1999) | No evidence | None identified | | |
| 8. Liver tumors | The incidence of liver tumors is increased in female mice exposed to 750 ppm (see Table 3, NTP 1999) | No evidence | Liver tumors are not significantly increased in male mice, or in rats of either sex (NTP 1999) | Human experience with phenobarbital indicates that this MOA is not relevant to humans (Holsapple et al. 2006, Elcombe et al. 2014) | Background rates for liver tumors are as follows: male mouse (~58%) > female mouse (~26%) > human (~0.9%, SEER 2014) ≈ male rat (~0%) ≈ female rat (~0%) |

Threshold events are possible.
- **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 correlation is observed between the incidence of eosinophilic foci and liver tumors in female mice (Figure 6); 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 (Engström 1984, Saghir et al. 2006, 2009). 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). Although data are lacking for CAR activation by ethylbenzene, its activation is inferred by the induction of CYP2B enzymes. 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, Elcombe et al. 2014). In addition, the phenobarbital-type liver tumor MOA only applies to non-genotoxic compounds, entirely consistent with the negative genotoxic profile of ethylbenzene. The incidence of thyroid gland follicular cell hyperplasia was significantly increased in male and female mice exposed to 750 ppm of ethylbenzene (NTP 1999), an effect that is also consistent with a phenobarbital-like alteration in thyroid hormone clearance (Meek et al. 2003).

- **Consistency of Association**—Ethylbenzene has been shown to enhance liver tumors in female 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), consistent with a role for the parent chemical in producing this tumor response.

- **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, 1999, Chan et al. 1998, Stott et al. 2003). On the other hand, liver tumors were absent in male mice following chronic exposure 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; Table 3), and therefore, this MOA explains some of the species- and sex specificity of observations made for liver tumors.

- **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 increased incidence of eosinophilic foci (NTP 1999, Chan et al. 1998). In a 13-week, subchronic inhalation study, mice exposed to 750 and 1000 ppm of
ethylbenzene 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 (Table 3) 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 of 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-year 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 with 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 after 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**—Carcinogenesis is recognized as a multistep process, in which cells acquire six capabilities: (1) sustaining proliferative signaling; (2) evading growth suppressors; (3) resisting cell death; (4) enabling replicative immortality; (5) inducing angiogenesis; and (6) activating invasion and metastasis (Hanahan and Weinberg 2000, 2011). The pattern of hepatic changes observed in mice is consistent with that observed for phenobarbital and the lack of liver tumors.

**Conclusion**

Confidence in the proposed MOA is medium since it is supported by available studies; however, there are some limitations in the available data (e.g., lack of studies that specifically address CAR activation by ethylbenzene). The key events in the MOA are summarized in Table 10. The proposed MOA is better supported by the available data than other potential MOAs (e.g., mutagenic MOA, see Table 11), and generally meets the modified Hill criteria for causation of liver tumors.
| Event                                                                 | Supporting evidence                                                                                                                                                                                                 |
|----------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1–3. Exposure/Absorption/Distribution                               | Events are the same as described in Table 9                                                                                                                                                                           |
| 4. Formation of DNA adducts/damage by ethylbenzene or metabolites   | No evidence                                                                                                                                                                                                         |
|                                                                      | A marginal increase in SCE was reported in human lymphocytes exposed \textit{in vitro} (Norppa and Vainio 1983). An increase in single-strand DNA breaks was reported in human lymphocytes (Chen et al. 2008) |
|                                                                      | No DNA adducts were detected in peripheral lymphocytes of exposed workers (Holz et al. 1995). Double-strand DNA breaks were not observed in human lymphocytes (Chen et al. 2008). Tests for biomarkers of DNA damage are generally negative for ethylbenzene (see Table 2) |
| 5. DNA miscoding resulting in gene mutation                         | A significant mutagenic response was reported in a mouse lymphoma assay. However this was only noted at the highest dose, and was accompanied by cytotoxicity (NTP 1999) |
|                                                                      | No evidence                                                                                                                                                                                                         |
|                                                                      | \textit{In vivo} genotoxicity studies are negative (NTP 1999, Mohitashamipur et al. 1985). In vitro genotoxicity studies are largely negative (see Table 2) |
|                                                                      | No DNA strand breaks or SCEs in exposed workers (Holz et al. 1995)                                                                                                                                                   |
| 6. Altered gene expression resulting in altered cell growth         | A positive response (\textasciitilde2-fold increase) was reported for cell transformation of Syrian hamster embryo cells, but was accompanied by reduced plating efficiency (Kerckaert et al. 1996) |
|                                                                      | No evidence                                                                                                                                                                                                         |
|                                                                      | None identified                                                                                                                                                                                                  |
| 7. Clonal expansion, uncontrolled growth, formation of preneoplastic lesions | NTP (1999) identified renal tubule hyperplasia in rats and eosinophilic foci in mice as preneoplastic lesions (Table 3)                                                                                                                                                 |
|                                                                      | No evidence                                                                                                                                                                                                         |
|                                                                      | None identified                                                                                                                                                                                                  |
| 8. Tumor formation, additional mutations leading to malignant behavior | Tumor observed in male rats (kidney and testes), female rats (kidney), male mouse (lung), and female mouse (liver) (NTP 1999, Table 3)                                                                                     |
|                                                                      | No evidence                                                                                                                                                                                                         |
|                                                                      | None identified                                                                                                                                                                                                  |
in female mice. The proposed MOA is supported by observations (altered foci) made in the key study (NTP 1999), as well as for structurally similar chemicals (oxazepam, pyrethrins, and fenbucanazole) that act via a phenobarbital-type MOA (Cunningham et al. 1994, Griffin et al. 1996, Parkinson et al. 2006, Jubb et al. 2006, Price et al. 2007). Since phenobarbital-type liver responses (key events 6–8 in Table 10) have been deemed not relevant to humans due to qualitative, as well as quantitative, species differences (Whysner et al. 1996, Holsapple et al. 2006, Elcombe et al. 2014), selection of an appropriate internal dose measure and low-dose extrapolation is not required. Consideration for an alternative MOA (direct genotoxicity) is provided below (following proposed MOAs for other observed rodent tumors).

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) exposure; (2) absorption; (3) distribution of ethylbenzene to lung; (4) metabolism to active metabolite; (5) possible oxidative stress secondary to high-dose glutathione (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 analogs (styrene, naphthalene, coumarin, cumene, alpha-methylstyrene, divinylbenzene, and benzofuran), 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, 2009, 2011). CYP2F2 activity is associated with club (Clara)-cell-specific CYP450 metabolites (Saghir et al. 2009), analogous to styrene lung toxicity MOA studies (Cruzan et al. 2002, 2009). These metabolites of ethylbenzene include 4-ethylphenol and 2-ethylphenol that are further metabolized to catechols, hydroquinones, and downstream quinone metabolites (Engström 1984, Midorikawa et al. 2004, Saghir et al. 2006). Cruzan et al. (2012) reported that CYP2F2 knockout mice failed to develop lung toxicity associated with styrene (necrosis, club cell exfoliation, and increased uptake of bromodeoxyuri-
dine) as observed in wild-type mice and CYP2E1 knockout mice. Metabolites of catechol and hydroquinone have been shown to auto-oxidize to protein-reactive and cytotoxic quinones (Rossi et al. 1986, Gant et al. 1988, O’Brien 1991, Tapper et al. 2000, Bus 2006). 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, 2010). 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**—An increase in male mouse lung tumors has been reported (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 (Engström 1984). Additionally, ethylbenzene has been shown to produce 4-ethylphenol and 2-ethylphenol in mouse lung microsomes (Saghir et al. 2006, 2009). 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 toxicity, 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 and club cells) that are known to be enriched in specific P450 (2F2) likely to contribute to ring oxidation of ethylbenzene. Lung toxicity in mice is specific to CYP2F2 activity for the structural analog, styrene (Cruzan et al. 2012). Since the incidence of hyperplasia/metaplasia was increased only in the sex and species (male mice) in which tumors were increased (Table 3), this MOA is consistent with the species- and sex-specific observations made for lung tumors in rodents.

• **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, 2009). These metabolites are further metabolized to catechols and hydroquinones. Metabolites of catechol and hydroquinone have been shown to auto-oxidize 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, 2009), which in turn could result in the further metabolism to catechols and hydroquinones. With respect to structurally similar chemicals (styrene and naphthalene), GSH depletion, oxidative stress, and cytotoxicity have been observed in mouse club 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 (Cruzan et al. 2009, 2012). However, CYP450 2F2 is a club-cell-specific enzyme involved in ethylbenzene metabolism and is present at high levels in mouse lung. Also, human lungs contain far fewer numbers of club cells than mice (Stott et al. 2003), and human lung microsomes, which instead contain CYP450 2F1, failed to or marginally metabolize ethylbenzene (Saghir et al. 2006, 2009). This observation is consistent with results obtained previously for structurally similar chemicals, 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 MOAs 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, 2009). The proposed MOA for lung tumors following styrene exposure is mediated by metabolites formed through the CYP2F2 enzyme (Cruzan et al. 2002, 2009, 2012), 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, have 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 club 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 12. 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, exist between humans and mice. For this current assessment, differences between CYP2F2 expression and activity are assumed to be quantitative in nature (see the next section).

Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?

Even though the qualitative impacts of the proposed MOA on tumor outcomes are not fully defined, quantitative differences between mice and humans should be considered: (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 club 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, 2009), an observation that is consistent with reports for chemicals (styrene, naphthalene, and coumarin) with a similar MOA (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 2014). Furthermore, large species differences (mouse>> rat > human) were observed for the formation of reactive metabolites of ethylbenzene (as indicated by binding to microsomal proteins) by lung microsomes via CYP2F2 and CYP2E1 activity (Saghir et al. 2010). Although a correlation has been reported between biomarkers of exposure to ethylbenzene (urinary mandelic acid) and biomarkers of oxidative stress (urinary 8-hydroxydeoxyguanosine) in spray painters, workers were also exposed to other chemicals (Chang et al. 2011). In contrast, significant correlations between biomarkers of exposure to ethylbenzene and oxidative stress were not observed in shipbuilding workers (Kim et al. 2011). 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.

Conclusion

Following exposure to ethylbenzene, the key events in the proposed MOA for male mouse lung tumors are summarized in Table 12. 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; Table 11) is provided below (following proposed MOAs for other observed rodent tumors).

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. Since 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 non-linear method of extrapolation (RfD approach) is indicated.

Proposed LCT 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. Rasoulpour et al. (2014) have suggested that there are nine potential MOAs for the production of LCT tumors in rats: 1) Mutagenicity; 2) Androgen receptor antagonism; 3) Estrogen receptor agonism/antagonism; 4) 5-Alpha-reductase inhibition; 5) Aromatase inhibition; 6) Reduced testosterone biosynthesis; 7) Increased testosterone metabolism; 8) Gonadotropin-releasing hormone (GnRH; luteinizing hormone-releasing hormone or LHRH) agonism; and 9) Dopamine agonism/enhancement.

A mutagenic MOA is relevant to human health, but is not supported for ethylbenzene (Table 11; see discussion below).
Table 12. Key events in the MOA for ethylbenzene-induced male mouse lung tumors.

| Event | Supporting evidence | Evidence in rodents | Evidence in humans | Potentially inconsistent evidence | Qualitative differences between rodents and humans | Quantitative differences between rodents and humans | Sources of non-linearity that may impact high-to-low dose extrapolation |
|-------|---------------------|---------------------|--------------------|-------------------------------|----------------------------------|----------------------------------|--------------------------------------------------|
| 1–3. Exposure/Absorption/Distribution | | Events are the same as described in Table 9 | | | | | |
| 4. Metabolism to active metabolite | Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of ethylbenzene (McMahon and Sullivan 1966, 1968, Engström 1984, Kaubisch et al. 1972, Stott et al. 2003, Saghir et al. 2006, 2009, Cossec et al. 2010) | Cytochrome P450-mediated reactions produce alkyl side chain and ring oxidation of EB (Engström et al. 1984, Sams et al. 2004, Saghir et al. 2006, 2009) | None identified | 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 et al. 2006, 2009) | Enzyme induction and metabolic saturation achieved above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation) |
| 5. 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) (Cruzan et al. 2002, 2009, 2012) | A correlation between urinary biomarkers of ethylbenzene exposure and oxidative stress has been reported, but also involves exposure to other chemicals (Chang et al. 2011) | None identified | None identified | None identified | None identified. Thresholds associated with depletion of tissue antioxidants |
| 6. Oxidation of macromolecules leading to cytotoxicity | Evidence in rodents is inferred from structurally similar chemicals (styrene) (Cruzan et al. 2002, 2009, 2011) | No evidence | None identified | None identified | None identified | None identified. Linearity is assumed |
| 7. Lung tumor formation | The incidence of lung tumors is significantly increased in male mice exposed to 750 ppm (see Table 3, NTP 1999) | No evidence | Lung tumors were not significantly increased in female mice, or in rats of either sex (NTP 1999) | None identified | Background rates for lung tumors are as follows: male mouse (~14%) > female mouse (~8%) ≥ human (~7%, SEER 2014) ≥ male rat (~6%) > female rat (~2%) | None identified. Linearity is assumed |
MOAs 2–7 are considered to be of low relevance to human health, and MOAs 8–9 are considered to be not relevant to human health (Rasoulpour et al. 2014). Limited data available for ethylbenzene best support an MOA involving increased testosterone metabolism (MOA 7), which is considered to be of low relevance to human health.

The key events in the proposed MOA include (1) exposure; (2) absorption; (3) distribution of ethylbenzene to the liver; (4) enzyme induction and increased testosterone clearance; (5) decreased serum testosterone levels; (6) increased luteinizing hormone level; (7) progression of Leydig cell hyperplasia; and (8) 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 non-monotonic, exhibiting a slight decrease in incidence at the lowest concentration before increasing at the middle and high concentrations, and is accompanied by reciprocal changes (e.g., negative trend) in the incidence of Leydig cell hyperplasia (Table 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). This negative correlation is possibly explained by the observation 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 1992a).

- **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**—Since a negative correlation was reported between the incidence of hyperplasia and LCT in male rats, and subchronic exposures to concentrations up to 1,000 ppm failed to produce testicular effects in rats (NTP 1992a), dose–response concordance is not supported for this MOA.

- **Temporal Relationship**—Induction of cytochrome P450 isozymes and increased testosterone hydroxylation have been observed in rat liver microsomes within 24–48 h after exposure to ethylbenzene (Yuan et al. 1997a,b); 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 1992a).

- **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 MOA that has been proposed for LCTs induced by oxazepam (Cook et al. 1999, Rasoulpour et al. 2014). The progression of Leydig cells to cancer involves a continuum of four mor-

![Figure 8. Correlation plot for LCT in rats and mice exposed to ethylbenzene.](image_url)
Rat Leydig Cells Are Sensitive to Prolactin—Rat Leydig cells possess additional receptors of prolactin (Zipf et al. 1982), which is not present in humans (Clayton and Huhtaniemi 1983). The absence of this protein in rats makes them more sensitive to perturbations in serum testosterone levels and subsequent effects.

Despite the presence of 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.

Although the data are limited and the precise MOA for the possible association between ethylbenzene exposure and LCTs in male rats is not known, it does not appear to involve genotoxicity (see discussion below; Table 11). Overall, there is insufficient information available to establish an MOA for LCTs in rats exposed to ethylbenzene with a high degree of confidence.

**Rat Leydig Cells Possess Additional Receptors**—Rat Leydig cells possess a receptor for GnRH (Cooke and Sullivan 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). Rasoulpour et al. (2014) identifies only a single MOA (mutagenicity) as being relevant to human health, which is not supported by the data for ethylbenzene (see discussion below; Table 11). All the remaining MOAs are either of low relevance or not relevant to human health. Limited data indicate that ethylbenzene may produce LCTs by perturbing testosterone metabolism. Based upon these fundamental differences between male rats and humans, the LCTs observed in rats are expected to be of low relevance 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, and nicotine) (Cook et al. 1999). Furthermore, hormonal imbalances and a number of clinical substances that cause increases in LCTs in rats have not resulted in an increased incidence of Leydig cell neoplasia in man (Capen 2001).

### Are key events in the animal MOA plausible in humans?

The key events for ethylbenzene producing LCTs in male rats are presented in Table 13. 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, Rasoulpour et al. 2014). 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 GnRH (Cooke and Sullivan 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).

Rasoulpour et al. (2014) identifies only a single MOA (mutagenicity) as being relevant to human health, which is not supported by the data for ethylbenzene (see discussion below; Table 11). All the remaining MOAs are either of low relevance or not relevant to human health. Limited data indicate that ethylbenzene may produce LCTs by perturbing testosterone metabolism. Based upon these fundamental differences between male rats and humans, the LCTs observed in rats are expected to be of low relevance 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, and nicotine) (Cook et al. 1999). Furthermore, hormonal imbalances and a number of clinical substances that cause increases in LCTs in rats have not resulted in an increased incidence of Leydig cell neoplasia in man (Capen 2001).

### 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-folds less sensitive than the 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 luteinizing hormone receptors, with rat cells possessing considerably more (~20,000/cell) than human cells (1500/cell) (Huhtaniemi 1983).**

- **The half-life of circulating luteinizing hormone differs between rats and humans, with rats exhibiting a lower half-life (5–10 minutes) compared with humans (> 100 minutes) (Caron et al. 1994, De Groot et al. 1995).**

- **Rats have a greater Leydig cell mass:volume ratio than 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).** Although a higher than expected fraction of surgically removed testicular tumors was reported to be LCTs (Leonhartsberger et al. 2011), the overall rate of testicular tumors (of which a fraction are LCTs) in the US population is very low compared with rats (5.6 cases per 100,000 or 0.0056%) (SEER 2014). 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 (1) exclusion of the only MOA relevant to human health (mutagenic); (2) the low relevance of proposed MOA to human health (Rasoulpour et al. 2014); (3) the high background response of Leydig adenomas in rats; (4) the non-monotonic nature of dose–response relationship for LCTs (Table 3); (5) the apparent lack of a dose–response effect on the incidence of Leydig cell hyperplasia and adenoma combined (i.e., only a dose-dependent shift in severity is observed, not incidence; Table 3); and (6) the absence of any increase in carcinomas; LCT is not considered an appropriate model for quantifying the potential risks to human populations.

### 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 on lesion severity by ethylbenzene exposure of Leydig cell hyperplasia, which is common to male rats. Although a precise MOA has not been established for ethylbenzene and LCT, a genotoxic MOA can be safely ruled out (see below “Consideration of direct genotoxicity as a default MOA for all tumor types”; Table 11). A possible MOA 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...
Table 13. Key events in the MOA for ethylbenzene-induced male rat LCTs.

| Event                                                                 | Supporting evidence                                                                 | Potentially inconsistent evidence | Qualitative differences between rodents and humans | Quantitative differences between rodents and humans | Sources of non-linearity that may impact high-to-low dose extrapolation |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------------------------|
| 1–3. Exposure/Absorption/Distribution                                | Events are the same as described in Table 9                                         | None identified                  | None identified                                   | None identified                                   | Enzyme induction and metabolic saturation above concentration of 500 ppm (tumor incidence was increased only at concentrations exceeding metabolic saturation) |
| 4. Enzyme induction resulting in increased testosterone clearance    | Induction of several P450 isozymes in rats, including those associated with testosterone metabolism (Yuan et al. 1997a, b) | No evidence                      | None identified                                   | None identified                                   | Potential threshold events                                        |
| 5. Decreased circulating levels of testosterone                      | Increased hydroxylation of testosterone in rat liver microsomes (Yuan et al. 1997a, b). Lower plasma testosterone levels are assumed. | No evidence                      | None identified                                   | Lack of sex-hormone-binding globulin in rats makes them more susceptible to serum testosterone perturbation than humans | None identified                                                   |
| 6. Increased circulating levels of luteinizing hormone               | No evidence                                                                         | No evidence                      | None identified                                   | Rat Leydig cells are prolactin sensitive, while human Leydig cells are insensitive to prolactin | None identified                                                   |
| 7. Progression of Leydig cell hyperplasia                           | Leydig cell hyperplasia showed a decreasing trend in rats (NTP 1999, see Table 3) | No evidence                      | None identified                                   | Quantitative differences in Leydig cell receptors and serum LH half-life (Rasoulpour et al. 2014) | None identified                                                   |
| 8. Leydig tumor formation                                            | The incidence of LCTs is in male rats exposed to ethylbenzene (NTP 1999, see Table 3) | No evidence                      | The incidence of LCTs is not increased in male mice exposed to ethylbenzene (NTP 1999) | None identified                                   | Background rate for LCTs approaches ~100% in rats (NTP 1999) and < 0.4% in humans (SEER 2014) | Potential threshold events |
Consideration of direct genotoxicity as an alternative MOA for all tumor types

Is the weight of evidence sufficient to establish the MOA in animals?

A direct genotoxic MOA was developed for all rodent tumor types based on a review of the general MOA proposed by Preston and Williams (2005). Under this MOA, the key events are assumed as follows: (1) exposure; (2) absorption; (3) distribution of parent chemical/metabolites to target tissues; (4) formation of DNA adducts/damage by ethylbenzene or metabolite(s); (5) miscoding of DNA nucleotide sequence resulting in a gene mutation; (6) alterations gene expression resulting in altered cell growth; (7) clonal expansion, uncontrolled growth, and formation of pre-neoplastic lesions; and (8) tumor formation with additional mutational events leading to tumor malignancy. The weight of evidence for this MOA is considered 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 2). 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, non-physiologic concentrations in which significant cytotoxicity (reduced growth) was observed. Ethylbenzene produced single-strand DNA breaks in human lymphocytes using an alkaline comet assay, but failed to produce double-strand DNA breaks in human lymphocytes using a neutral comet assay (Chen et al. 2008). Ethylbenzene-induced damage was inhibited by free radical scavengers (5,5’-dimethyl-pyrrrole-n-oxide and N-tert-butyl-phenylnitron), and the authors suggested that the effects were attributable to the formation of oxidative damage (8-oxo-guanine formation). 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 that 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, and 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.

- **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 intraperitoneal [ip] injection). The few positive results for the genotoxic-
ity 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 1061.6 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 non-lethal 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 of 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 ip injection of ethylbenzene resulted in no increase in micronuclei (Mohtashamipur et al. 1985). Occupational exposure (8-h 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). Since 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, 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 11. In summary, all in vivo studies have been negative for genotoxicity and the in vitro studies have been predominantly negative for genotoxicity. NTP concluded “Ethylbenzene gave little indication of mutagenicity, in vitro or in vivo” (NTP 1999). Direct genotoxicity does not seem to be a supportable MOA for ethylbenzene-induced kidney, liver, or lung tumors in either rats or mice, and therefore is not thought to support decisions made in the cancer dose–response assessment.

**Cancer dose–response assessment**

A cancer dose–response assessment has not been prepared by US EPA because at the time of the assessment (US EPA 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, including the selection of the following: (1) Data Set; (2) Dose Measure and Response Measure; (3) Dose–Response Model; (4) Point of Departure; and (5) Low-Dose Extrapolation Method. Each of these decisions is summarized below.

**Cancer 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, liver, and LCT. Based upon a consideration of the MOAs summarized above, 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. LCTs were excluded from the quantitative assessment, since the proposed MOA has low relevance to human health, the increase in incidence was weak and non-monotonic, a dose–response effect on the incidence of Leydig cell hyperplasia and adenoma combined is lacking (Table 3), and no increase in carcinomas were observed. 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 14.

**Cancer dose measure**

Based upon the MOA described above 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. Since 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 14). Details of the mouse PBPK model are found in Nong et al. (2007).

**Table 14. Dose–response data for lung tumors observed in mice exposed to ethylbenzene.**

| Concentration (ppm) | 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     |
Cancer 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.

Cancer 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 15 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 9).

Cancer 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. The 10% benchmark response rate serves as the default point of departure as described by US EPA guidelines (2005b).

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 103,000 mg metabolized in lung/kg lung/week, respectively.

Extrapolation to low doses and potentially susceptible subpopulations

Based upon a consideration of the MOA described above, the dose–response relationships for ethylbenzene-induced lung and liver tumors are expected to be non-linear 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.

Table 15. Comparison of models fit to ethylbenzene lung tumor data.

| Model              | 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   |

Cancer values

Cancer values for ethylbenzene based upon non-linear 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 343 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 (see Supplementary Material, Supplement A) to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157**—The PBPK model for ethylbenzene with a human lung metabolism estimate derived from the analytical detection limit for microsomal incubations (see Supplementary Material to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157) 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 = 13.4 ppm. The corresponding daily ingestion rates are 7.1, 4.3, and 18 mg/kg bw/day, respectively.
- **Using a conservative estimate of human lung metabolism rate (see Supplementary Material to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157**—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.
- **UFH**—In the absence of specific information on human variation, a default factor of 10 was considered to be appropriate for ethylbenzene.
- **UFD**—Since 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 (3 × 10 × 10 × 1 × 1).
with a human lung metabolism estimate derived from the rat lung $V_{\text{max}}$ determined using rat lung microsomes (see Supplementary Material to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157; Saghir et al. 2007, 2009) 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 = 2.0 ppm. The corresponding daily ingestion rates are 1.1, 0.71, and 2.9 mg/kg bwt/day, respectively.

Toxicity reference value summary

A chronic non-cancer RfC of 0.3 ppm is proposed for use in this assessment, 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) (US EPA 1991).

A chronic non-cancer 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 = 2.0 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 = 2.9 mg/kg bwt/day).

Exposure assessment

Introduction

The major objectives of both the Tier 1 VCCEP exposure assessment for ethylbenzene (ACC 2007) and this updated exposure assessment are to

- Document sources and significant pathways of exposure for children and prospective parents;
- Develop central tendency and upper-bound exposure estimates for each pathway based upon generally conservative assumptions (tending to overestimate rather than underestimate potential exposures); and
- Distinguish, on a semi-quantitative basis, that proportion of each exposure pathway that is directly attributable to the ethylbenzene/styrene chain of commerce.

As discussed in the following sections, ethylbenzene emissions and ambient concentrations continue to decline significantly. Additional high-quality population-based personal exposure data have become available, and there have been substantive changes in relevant EPA exposure and toxicity assessment guidance. Together, these data and guidance changes serve to both simplify and reduce uncertainty in estimates of ethylbenzene exposures and potential health risks to children and prospective parents.

Literature search strategy

The online databases PubMed and Google Scholar were searched to identify English-language publications in the scientific literature (peer-reviewed articles and academic theses) pertaining to ethylbenzene exposure. Emphasis was placed on North American publications, but data from Western European countries were also reviewed. Relevant data and reports produced by U.S. (ATSDR, USEPA, US Food and Drug Administration [FDA], and National Library of Medicine or NLM), Canadian (Health Canada and Environment Canada), and international (WHO and International Agency for Research on Cancer or IARC) governmental agencies were identified through website searches. Manual searches were conducted based on lists of citations in publications and governmental documents.

Environmental releases of ethylbenzene

Fugacity modeling indicates that over 98% of ethylbenzene in the environment partitions to the atmosphere, where it exists predominantly in the vapor phase until it is removed through physical processes such as partitioning into clouds or rainwater, and by chemical transformations (ACC 2007, Section 5.2.2.1). It does not adhere to soil or sediment, and is not expected to bioaccumulate in food chains.

In 2009, the Chemical Economics Handbook (CEH) listed seven US producers of ethylbenzene, one of which (Dow Chemical, USA) planned to cease ethylbenzene and styrene production by the end of 2009 (CEH 2009). As shown in Table 16, the combined production of these manufacturers was 13,409 million pounds, a 7% decrease compared with the 2002 data presented in the VCCEP submission (ACC 2007, Table 5–1). Ethylbenzene emissions data for six of the eight manufacturing facilities listed in Table 16 were identified in the 2009 Toxics Release Inventory (TRI) database and compared with total ethylbenzene emissions that year. The TRI, a publicly available database maintained by the EPA since 1986,
contains self-reported annual information on toxic chemical releases and waste management activities from certain industries as well as federal facilities. Relevant TRI data limitations include the two-year time lag between toxic release and data release; reporting exemptions based on size, primary business activity, chemical manufacturing and processing, and use thresholds; and potential inaccuracies in self-reported data (US EPA 2014c). As in the Tier 1 VCCEP submission (ACC 2007, Section 5.3.2), the bulk of ethylbenzene release from these producers is via deep well injection, with little or no possibility for human exposure (Table 17, an update of Table 5–12 in ACC [2007]).

As in 2002 (ACC 2007, Section 5.3) and 2013, TRI data (US EPA 2014b) indicate that the major route of industrial ethylbenzene emission is release to air. Total releases of ethylbenzene from industrial facilities decreased more than twofold from 2002 to 2013 (Figure 10, an update of Figures 5–6 and 5–7 in ACC [2007]). In terms of industry category, the top three contributors to ethylbenzene releases in 2013 were the same as those in 2002—chemicals, transportation equipment, and petroleum (Figure 11, an update of Table 5–11 in ACC [2007]).

The 1999 National Emission Inventory (NEI) database available at the time of the Tier 1 VCCEP submission indicated

| Type of release | All US facilities | Ethylbenzene producers |
|-----------------|-------------------|------------------------|
|                 | % Total | % Total |
| **On-site disposal and release** | | |
| Underground Injection Class I Wells | 0.50 | 0.49 |
| RCRA Subtitle C Landfills | 2.2 × 10^{-6} | 1.0 × 10^{-6} |
| Other On-Site Landfills | 2.1 × 10^{-4} | 9.4 × 10^{-5} |
| Fugitive Air Emissions | 1.1 | 7.5 × 10^{-2} |
| Point Source Air Emissions | 1.4 | 6.9 × 10^{-2} |
| Surface Water Discharges | 3.0 × 10^{-3} | 1.0 × 10^{-5} |
| Total On-site Disposal or Other Releases | 3.0 | 0.64 |
| **Off-site disposal and release** | | |
| Underground Injection to Class I Wells | 1.4 × 10^{-2} | 1.0 × 10^{-6} |
| RCRA Subtitle C Landfills | 9.9 × 10^{-2} | 2.4 × 10^{-5} |
| Other Landfills | 4.6 × 10^{-2} | 0.0 |
| Storage Only | 9.4 × 10^{-2} | 6.0 × 10^{-2} |
| Total Off-site Disposal or Other Releases | 0.28 | 6.0 × 10^{-2} |
| Total On-/Off-site Disposal or Other Releases | 3.3 | 0.7 |

*Data from TRI (http://iaspub.epa.gov/triexplorer/tri_release.chemical (accessed December 2013) in 10^6 lb.

2009 TRI data for Americas Styrenics, Cos-Mar, Dow, INEOS NOVA (facility in Alvin, TX), Lyondell, and Westlake (see Table 16).

Figure 10. Trends in total ethylbenzene releases to air, water, and land 1988–2013 (from TRI database).
that mobile sources contributed the majority of ethylbenzene emissions at nearly 76%, followed by non-point (area) sources at 19%. The smallest contribution in the 1999 NEI database came from major point sources (5%). Industries under Standard Industrial Classification (SIC) code series 2800 (Chemicals and Allied Products) and 3000 (Rubber and Miscellaneous Plastics Products) contributed 12% to total point-source emissions, and less than 1% to total ethylbenzene emissions (ACC 2007, Figure 5–5). On this basis, it was conservatively estimated that 1% of airborne ethylbenzene exposure was derived from the ethylbenzene/styrene chain of commerce (ACC 2007, Section 6.7.1.2).

There was a 14% reduction in ethylbenzene emissions between the 2005 and 2008 NEI, including an 11.5-million-pound reduction from industrial processes (US EPA 2013a). In Version 1 of the 2011 NEI database (NEIv1, released September 30, 2013, US EPA 2013b), total ethylbenzene emissions decreased by 19% from 2008 (151.1 vs. 186 million pounds in 2011 and 2008, respectively). Mobile sources contributed 80% (121 million pounds) to total ethylbenzene emissions in NEIv1 2011, while industrial processes contributed less than 3% (3.7 million pounds) (Figure 12). Only 0.1 million pounds (0.07%) came from all facilities potentially within the ethylbenzene/styrene chain of commerce (North American Industry Classification System [NAICS] codes 325211 [Plastics Material and Resin Manufacturing], 325212 [Synthetic Rubber Manufacturing], and 326140 [Polystyrene Foam Product Manufacturing]) in 2011—nearly ten times lower than the percent contribution of the SIC code series 2800 and 3000 (0.6%) estimated in the Tier 1 VCCEP submission (ACC 2007, Section 6.7.1.2).
This difference is likely due primarily to the more granular nature of the newer NAICS classification system. Although this lower contribution by the ethylbenzene/styrene chain of commerce to overall ethylbenzene emissions may well be valid, these results are preliminary as the 2011 NEIv1 database will likely undergo further revision. Therefore, the 1% contribution to ambient ethylbenzene levels by the ethylbenzene/styrene chain of commerce applied in the Tier 1 VCCEP submission is conservatively retained for this updated exposure assessment.

Additional sources of ethylbenzene identified in the 2011 NEIv1 database are waste disposal, combustion of natural materials (wood and other fuels) or from incinerators burning various hydrocarbon-containing waste streams, bulk gasoline terminals, commercial cooking, and agriculture. Ethylbenzene is also a constituent of cigarette smoke (Pankow et al. 2004, Charles et al. 2007, 2008, Polzin et al. 2007), a source not included in the NEI. Thus, ethylbenzene is released both directly to smokers and indirectly to non-smokers through environmental tobacco smoke (ETS). In the Tier 1 VCCEP submission, ethylbenzene emissions from tobacco products was estimated at around 0.11 million pounds based on 2004 tobacco consumption estimates (ACC 2007, Section 5.3.3). In 2012, the CDC estimated that total consumption of all combustible tobacco had decreased to 326.6 billion cigarette equivalents (CDC 2012). Assuming the same ethylbenzene content in each cigarette (130 μg), the revised estimate of ethylbenzene emission from tobacco smoke is reduced to about 0.09 million pounds—a similar order of magnitude as 2011 emissions by ethylbenzene producers.

This brief comparison of previous and current data supports the conclusions that (1) ethylbenzene releases from industrial and other sources have been substantially lowered over the past decade, and (2) ethylbenzene exposure directly connected with the chain of commerce, including exposures from production and use of ethylbenzene as a neat compound, remains a minor contributor to total ambient exposure. As such, current potential exposure to children and prospective parents not employed as ethylbenzene production workers is expected to remain dominated by “out-of-chain” sources such as automobile exhaust and tobacco smoke.

**Exposure scenarios**

The physicochemical characteristics and behavior of ethylbenzene in the environment indicate that the most likely route of human exposure is inhalation. Widespread human exposure can also occur via ingestion of food items into which atmospheric ethylbenzene has partitioned.

The strategy used to evaluate exposures to ethylbenzene involved elements of both chain of commerce and receptor-centered approaches, as summarized in Table 18. Individuals exposed by way of the ethylbenzene/styrene chain of commerce include (1) workers involved in ethylbenzene production and styrene/polystyrene manufacture, and (2) the general public exposed via (a) ambient environmental releases from these industries and (b) migration or off-gassing from styrenic products. The following receptor populations were therefore examined for potential exposure to ethylbenzene in the ethylbenzene/styrene chain of commerce (ACC 2007):

- 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 consuming foods containing styrenic materials; and
- 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., and traveling in motor vehicles (i.e., the general public).

Potentially complete exposure pathways evaluated for these groups are summarized in Figure 13 and Table 19. 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 of production workers and their families were limited to direct inhalation while at work and partitioning to mother’s milk, respectively. Members of the general public were assumed to be exposed to ethylbenzene via the diet, but dietary intake was not considered for production workers because it was assumed that its contribution would be very small in comparison with workplace exposure. As discussed in the original VCCEP submission (Section 6.2.2.2), while groundwater and surface water resources can be affected by localized spills and other releases containing ethylbenzene, available data consistently indicate low detection frequency and concentrations well below the MCL in both groundwater and surface water used for drinking (ACC 2007, Rowe et al. 2007, Carter et al. 2008, US EPA 2010). Therefore, intake by way of potable water (via ingestion, inhalation, and/or dermal contact) is considered negligible and is not considered in this exposure assessment.

The only exposure pathways unique to young children were ingestion of mother’s milk and mouthing of styrenic toys. Potential exposure to children mouthing plastic toys (which are usually composed of polyvinyl chloride rather than styrene-containing polymers) was conservatively estimated for children aged 2 to 36 months in the Tier 1 VCCEP submission as $6.8 \times 10^{-10}$ to $1.4 \times 10^{-7}$ mg/kg-day, depending on age and exposure assumptions (average or maximum) (ACC 2007, Table 6–54). Since these daily exposure levels were 4–5 orders of magnitude lower than those associated with the dominant inhalation exposure pathway (ACC 2007, Section 6.7.4.1), this potential exposure pathway was considered very minor and not included in the present exposure assessment.

With the exception of workplace air concentrations, data are not available to clearly distinguish the original source(s)

| Table 18. 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 | Personal exposure via inhalation | Diet (food, formula, and breastfeeding) |

| • 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 consuming foods containing styrenic materials; and |
| • 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., and traveling in motor vehicles (i.e., the general public). |
of ethylbenzene levels in air and food. Therefore, the approach taken in this assessment was to apply emission contribution estimates and 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. As discussed previously, the 1% contribution to ambient ethylbenzene levels by the ethylbenzene/styrene chain of commerce applied in the Tier 1 VCCEP submission is conservatively retained for this updated exposure assessment.

An upper-bound estimated contribution to food items of 25% was derived from kinetic modeling of potential ethylbenzene migration from food packaging in the Tier 1 submission (ACC 2007, Section 6.4.3.2). As very similar results were obtained in the updated food evaluation, the assumption that 25% of ethylbenzene in the diet may be associated with the ethylbenzene/styrene chain of commerce was also retained in the present assessment.

Concentrations of ethylbenzene in potential exposure media

Inhalation

When the Tier 1 VCCEP exposure assessment for ethylbenzene was prepared in the mid-2000s, it was recognized that indoor air concentrations of ethylbenzene and other VOCs are consistently higher than outdoor concentrations, and that personal exposures are usually higher than—and imperfectly predicted by—inhalation measurements. However, the only nationally representative data available were ambient monitoring data. Therefore, the approach taken in the Tier 1 VCCEP submission to estimate time-weighted ethylbenzene concentrations in important indoor microenvironments (homes, schools, offices, and motor vehicles) was to apply indoor/outdoor ratios estimated from the literature to central tendency and upper-bound urban and rural outdoor air concentrations. An additional multiplier was used for the increased exposure of smokers and those exposed to ETS.

A number of large studies examining indoor and personal exposures to VOCs in North America, Western Europe, and Australia have been published since 2007, and the CDC has added VOCs to the analytes biomonitored in the general US population in the continuous NHANES. As discussed in the following sections, these studies have provided much high-quality data concerning exposure sources and magnitudes, and confirmed the preferability of personal over environmental—outdoor or indoor—measurements for ethylbenzene exposure estimation.

Table 19. Exposure pathways examined for representative populations.

| Age group (years) | Styrene chain of commerce | Other sources |
|-------------------|---------------------------|--------------|
|                   | Inhalation                | Ingestion    | Inhalation | Ingestion |
|                   | Workplace | Personal exposure | Mother’s milk | Food | Personal exposure | Ingestion | Food |
| < 1 (breast-fed)  | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| < 1 (bottle-fed)  | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 1 < 2             | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 2 < 3             | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 3 < 6             | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 6 < 11            | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 11 < 16           | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| 16 < 21           | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| Adult public      | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
| Adult worker      | ●                     | ●             | ●           | ●    | ●             | ●         | ●      |
Outdoor air

Human exposure to ethylbenzene and other VOCs has traditionally been estimated based on abundant data from fixed-site ambient air quality monitoring networks, typically annual average concentrations. Data have consistently demonstrated a causal relationship between motor vehicle emissions and ambient ethylbenzene concentrations (Wallace et al. 1987b, Mohamed et al. 2002, Batterman et al. 2002, Spengler et al. 2011, Fujita et al. 2011, Miller et al. 2012). EPA’s AirData database provides yearly summaries of data collected from fifty states, the District of Columbia, Puerto Rico, and the US Virgin Islands (http://www.epa.gov/airquality/airdata/). Average annual ethylbenzene concentrations reported for the years 1984 through 2014 are summarized by monitoring location type (urban, suburban, and rural) in Figure 14. Concentrations of ethylbenzene were higher in urban and suburban than in rural areas, as expected due to its association with automotive fuels and exhaust.

While the annual average ethylbenzene concentrations have remained quite consistent in rural areas, data collected in urban and suburban areas demonstrated a decreasing trend (about 5% annually from 1990 to 2005 [McCarthy et al. 2007]). These decreases paralleled the decreasing trend observed in on-road motor vehicle emissions of ethylbenzene over the same decade (Cook et al. 2004, Figure 14). The decreasing trend continued from 2006 to 2014 (Figure 14). In EPA’s 2012 Urban Air Toxics Monitoring Program or UATMP (US EPA 2014a), ethylbenzene was detected in 1452 of 1459 valid samples (99.5%) at concentrations ranging from 0.02 to 3.6 micrograms per cubic meter (µg/m³). The mean, median, and standard deviation of this data set were 0.35 µg/m³, 0.24 µg/m³, and 0.36 µg/m³, respectively (US EPA 2014a). In 2014, the average ethylbenzene concentration in rural areas was 0.05 µg/m³ compared with 0.16 and 0.31 µg/m³ at suburban and urban stations, respectively. On-road motor vehicle emissions of ethylbenzene also continued to decline through 2011, the most recent year for which NEI results are currently available (Figure 14).

Microenvironmental indoor air

Extrapolating data from ambient monitoring sites is appropriate for broadly assessing the exposure of large populations to chemicals in outdoor air. However, it arbitrarily homogenizes exposure concentrations and, more critically, fails to capture indoor and personal exposures.

Indoor air quality is a complex function of a building’s location, characteristics, composition, contents, and uses (e.g., Kim et al. 2001). The TEAM studies (Wallace et al. 1987b), conducted in eight urban areas in the 1980s, were the first major US exposure assessment to measure indoor air and individual personal exposures (measurement of airborne chemicals in a person’s breathing zone) and biomonitoring in breath within a probability-based sampling framework. An important finding from the TEAM studies was that for many of the sampled VOCs, including ethylbenzene, outdoor concentrations were considerably lower than indoor concentrations, which were in turn lower than personal concentrations. Thus, personal exposures to ethylbenzene and other common VOCs are often

![Figure 14. Trends in annual average ethylbenzene concentrations in ambient air and amount emitted from motor vehicles.](image-url)
underestimated by not only outdoor but also indoor measurements (Wallace et al. 1985, Wallace and Pellizzari 1986a, Wallace et al. 1986b, 1987a, 1987b, 1988, 1989, 1991, Wallace 1991a, 1991b).

The indoor environment became the major venue for exposure assessment not only because concentrations of most VOCs are typically higher in buildings than in ambient air, but also because most individuals, including children, spend the majority of their time indoors (Klepeis et al. 2001). Sampling of personal concentrations in the breathing zone throughout an individual’s daily activities provides a highly accurate estimate of inhalation exposure to VOCs because it integrates exposures in all areas, and accounts for their individual characteristics and varying activities. However, measuring personal exposures for a large number of people (including potentially vulnerable groups of particular interest, such as children and the elderly) is impractically intrusive, time-consuming, and expensive. An individual’s personal exposure over a particular time period depends on (1) chemical concentrations in the indoor and outdoor microenvironments (such as home, schools, offices, stores and restaurants, and motor vehicles) moved through during routine daily activities, (2) the time spent in each of these microenvironments, and (3) the level of activity in each microenvironment. Thus, many recent studies have relied on microenvironmental monitoring combined with time/activity budgets to (1) achieve more accurate exposure quantitation, and (2) identify determinants of exposure for individuals with particularly high or low exposure and other subgroups of interest (Edwards et al. 2006, Dodson et al. 2007, Sexton et al. 2007, Jia et al. 2008a, Ashmore and Dimitrulopoulou, 2009, Harrison et al. 2009, Johnson et al. 2010, Su et al. 2013, Cometto-Muñiz and Abraham 2015).

Other large-scale investigations in North America, Western Europe, and Australia that have provided much information about the sources and magnitude of exposure to ethylbenzene and other VOCs in the indoor environment include the National Human Exposure Assessment Survey or NHEXAS (Gordon et al. 1999); the Air Pollution Exposure in European Cities or EXPOLIS study (Edwards et al. 2001a, 2001b, 2005, 2006); the 1999–2000 NHANES (Lin et al. 2007, Jia et al. 2012, CDC 2013) the 2001–2002 Syracuse Assessment of Urban Dwellings for Indoor Toxics or AUDIT study (Zhang et al. 2003); the School Health Initiative: Environment, Learning, and Disease or SHIELD study (Adgate et al. 2004, Sexton et al. 2004, 2005); the Toxics Exposure Assessment Columbia-Harvard or TEACH study (Kinney et al. 2002, 2005, 2008, Sax et al. 2004, 2006); the Detroit Mechanistic Indicators of Childhood Asthma or MICA study (Johnson et al. 2009, 2010); the Boston Exposure Assessment in Microenvironments or BEAM study (Dodson et al. 2007, 2008); the European Indoor Air Monitoring and Exposure Assessment Project or AIRME (Kotzias et al. 2009); Critical Appraisal of the Setting and Implementation of Indoor exposure Limits in the EU (the INDEX project) (Kotzias et al. 2005); the Relationship of Indoor Outdoor and Personal Air (RIOPA) study (Weisel et al. 2005, Kwon et al. 2006, Su et al. 2012, 2013); Health Canada’s indoor air quality studies (Zhu et al. 2005, Stocco et al. 2008, Héroux et al. 2008, 2010, Health Canada 2010a&b, 2013, Wheeler et al. 2013); the Detroit Exposure and Aerosol Research Study or DEARS (Williams et al. 2009, Chin et al. 2014); and the Measurement and Modelling of Air Toxic Concentrations for Health Effect or MATCH studies (Delgado-Saborit et al. 2009, 2011).

As with outdoor levels of ethylbenzene, indoor levels have decreased since around 1990 (Hodgson and Levin 2003, Dawson and McAlary 2009, US EPA 2011a, Zhu et al. 2013). However, the distribution of ethylbenzene in residential indoor air derived from a variety of studies has not changed significantly in the past decade (Figure 15). Dawson and McAlary (2009) combined statistics from studies of North American residential indoor air (non-smoking) published between 1990 and 2005, by computing the means of the percentiles reported in individual studies, weighted by sample size. Table 20 shows their summary statistics for ethylbenzene as well as similar statistics computed from ethylbenzene data set presented by US EPA (2011a) and a more current set of studies (1999–2011) compiled for the present paper (Supplementary Material, Supplement B to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Tables B-1 and B-2). No distinction is made as to geographic location, housing type, or setting (urban, suburban, and rural). The fact that the majority of studies were conducted in urban areas suggests that these distributions are a conservative representation of US population exposure.

Available data indicate that factors that contribute to ethylbenzene levels in residences (and other buildings) include (1) automotive sources (traffic density and the presence of attached garages or structures where gasoline or kerosene may be stored); (2) ETS; (3) the presence or use of certain household products or hobby materials containing mixed xylenes; and (4) styrene building and furnishing materials. As discussed in the VCCEP assessment (ACC 2007, Sections 6.3.1.2 and 6.3.1.3), household chemicals and styrene construction and consumer products are relatively minor contributors to ethylbenzene concentrations in indoor air. The dominant source of ethylbenzene in residential indoor air, as in outdoor air, is motor vehicle emissions. TEAM and numerous subsequent studies have found ethylbenzene concentrations in indoor air to be significantly increased by local traffic volume, driving, and being in or near a garage or pumping gas. In urban environments, the contribution from these sources evidently obscures that from indoor sources, including ETS; household chemicals, building materials, and furnishings; and household electronic devices (Wallace et al. 1985, 1987a&b, 1988, 1991, Anderson et al. 2001, Edwards et al. 2001a, Ilgen et al. 2001, Kim et al. 2002, Weisel et al. 2005, Edwards et al. 2006, Batterman et al. 2006, 2007, Kwon et al. 2006, Hinwood et al. 2007, Sexton et al. 2007, Dodson et al. 2008, Jia et al. 2008b, Harrison et al. 2009, D’Souza et al. 2009, Symanski et al. 2009, Wang et al. 2009, Miller et al. 2010, Hun et al. 2011, Liou et al. 2011, Lim et al. 2014). Most (but not all) air monitoring data indicate that smoking is associated with higher indoor concentrations of ethylbenzene (Heavner et al. 1995, 1996, Hodgson et al. 1996, Anderson et al. 2001, Edwards et al. 2001b, Kim et al. 2001, Xie et al. 2003). Also, summarized data from studies of North American homes with smokers are shown in Table 20 (Supplementary Material, Supplement B to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Table B-2).

A detailed study of U.K. urban, suburban, and rural non-smoking adults’ exposures to air toxics (Harrison et al. 2009)
provided a representative picture of the contribution of various microenvironments to ethylbenzene exposure, with and without exposure to ETS (Figure 16). Not surprisingly, ethylbenzene exposure comports closely with Klepeis et al.’s (2001) estimates of the time spent in major microenvironments. Similar results were obtained in a recent analysis of exposure determinants in the US RIOPA study (Weisel et al. 2005), which examined non-smoking adults and children in three urban areas with diverse sources of air toxics: Elizabeth, NJ (part of an urban corridor, with a high population density, heavy traffic, and multiple point sources); Houston, TX (heavy traffic, as well as the highest density of petrochemical facilities in the world); and Los Angeles County (vehicular emissions from major freeways). Su et al. (2013) reported that home ethylbenzene levels dominated median personal exposures (64–73% of total personal exposure). Their results also confirm the seemingly paradoxical fact that motor vehicles are the primary source of ethylbenzene in indoor air: the outdoor source exposure fraction was 48% versus 15% for indoor sources (Su et al. 2013). Similar results were obtained for other VOCs lacking strong indoor sources (Su et al. 2013).

Production facilities

According to the OECD Screening Initial Data Set or SIDS for ethylbenzene (OECD 2002), 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 US manufacturers of ethylbenzene conducted by the Styrene and Ethylbenzene Association or 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 (433–4333 μg/m$^3$) for an 8-h time-weighted average (TWA) (Helmes 1990, as cited in ATSDR 1999).

Seven US ethylbenzene producers compiled worker exposure data collected between 1990 and 2000 (ACC 2001 [ACC

Table 20. Summary statistics for indoor air concentrations of ethylbenzene measured in North American residences (μg/m$^3$).

| Study years | N   | 25 | 50 | 75 | 90 | 95 | Max | Source                        |
|-------------|-----|----|----|----|----|----|-----|-------------------------------|
| 1981–2004   | 2298| 1.6| 2.7| 5.2| 9.9| 15.2| 185.6| US EPA (2011a)               |
| 1990–2005   | 1484| 0.8| 2.0| 3.0| 8.6| 14.0| 126.0| Dawson and McAlary (2009)    |
| 1997–2003   | 400 | 0.4| 1.0| 2.8| 7.4| –   | –   | NYSDOH (2005)                |
| 1999–2010   | Various | 0.5 | 1.5 | 2.8 | – | 9.0 | – | Logue et al. (2011)*          |
| 1999–2007   | 327 | 1.1 | 2.5 | 2.0 | 13.3 | 19.0 | 451.6 | Present study (smoking)**    |
| 1999–2011   | 8084| 0.7 | 1.7 | 2.7 | 7.5 | 13.5 | 159.2 | Present study (non-smoking)*** |

– not provided.
* Includes some European and Asian data.
** Some studies included smoking households. Data and sources provided in Supplementary Materials Table B-1.
*** Data and sources provided in Supplementary Materials Table B-2.

Figure 15. Weighted average 25th, 50th, and 95th percentiles of ethylbenzene concentrations in North American non-smoking residences, 1999–2010.
There were a total of 1727 personal monitoring samples (eight hours, TWAs) representing exposures for process operators, maintenance workers, loading/unloading, quality laboratory workers, and supervisory/professional workers. As shown in Table 21, approximately 71% of the measurements were either non-detectable or less than 0.1 ppm (434 μg/m³), 25% were between 0.1 ppm and 1.0 ppm (4343 μg/m³), 4% were greater than 1.0 ppm and less than 5 ppm (21 714 μg/m³), 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 μg/m³), and the other two results were unspecified. All of these values are very low compared with the U.S. Occupational Safety and Health Administration’s chronic occupational exposure standard for ethylbenzene of 100 ppm (433 400 μg/m³), also promulgated as occupational guidelines by the American Conference of Governmental Industrial Hygienists (ACGIH) and the National Institute of Occupational Safety and Health (NIOSH) (CDC/NIOSH 2007). The ACGIH has more recently adopted an occupational exposure limit of 20 ppm for ethylbenzene (ACGIH 2011). These data were considered appropriate for characterizing industrial exposure. The majority (71%) of the workers in this study were exposed to less than 0.1 ppm (434 μg/m³); 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 (4343 μg/m³), which was therefore selected as the upper-bound exposure estimate.

### Personal exposure

Although microenvironmental studies have provided a wealth of information regarding exposure levels and sources, it is virtually impossible to identify and measure concentrations in all potentially important microenvironments. A number of high-quality studies have measured personal exposure concentrations in targeted populations or convenience samples, but they are not necessarily representative of the general US population. The only available personal exposure study that is nationally representative is the NHANES VOCs Study, in which a subsample of 851 adults (aged 20–59 years) out of the overall NHANES sample wore badge-type passive exposure monitors for 48–72 h (Jia et al. 2008c). Jia et al. (2008c) selected 665 valid results from this data set and weighted them to derive national-level VOC exposure statistics. This data set

| Job description            | Non-detectable | <0.1† | >0.1–1.0‡ | >1.0–5.0¶ | >5.0§ |
|----------------------------|----------------|-------|-----------|-----------|-------|
| 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   |

*Source: American Chemistry Council (2001) (ACC 2007, Appendix J).
†<433 μg/m³.
‡>433–4343 μg/m³.
¶>4343–21 714 μg/m³.
§>21 714 μg/m³.
is considered the best available for characterization of ethylbenzene exposure to the general US adult population, and so is used as the primary basis for quantifying ethylbenzene exposure in this assessment.

Central tendency and upper-bound personal exposure estimates for non-smoking adults from this data set are the geometric mean (2.9 µg/m³) and 90th percentile (14.2 µg/m³), respectively (Table 22). These values are conservative, because approximately 27% of NHANES subjects were determined to be smokers or have regular exposure to ETS based on serum cotinine levels. The effect of smoking may be reflected in the fact that summary data from two contemporaneous (Payne-Sturges et al. 2004, Weisel et al. 2005) and two more recent non-smoking adult personal exposure studies are generally lower (Dodson et al. 2007, Lioy et al. 2011) (Supplementary Material, Supplement B to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Table B-3).

Comparing personal ethylbenzene exposure levels in smokers versus non-smokers in the NHANES data set, Symanski et al. (2009) reported a weighted geometric mean for smokers of 3.6 µg/m³. This value is used to represent the central tendency personal ethylbenzene exposure concentration for smoking adults (Table 22). Effects of smoking on upper quantiles of the distribution were not reported. However, as these higher percentiles likely include a majority of smokers, further adjustment of the upper-bound personal exposure estimate for smoking is not considered necessary.

Several studies have focused on children’s personal inhalation exposure to ethylbenzene (Supplementary Material, Supplement B to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Table B-3). However, only the RIOPA study directly compared personal exposures in cohabiting children and adults (Weisel et al. 2005). In the RIOPA study, personal concentrations of ethylbenzene and other air toxics for a convenience sample of 309 non-smoking adults (aged 20–89 years) and 118 children (aged 6–19 years) in three urban areas. Each participant was sampled twice. A comparison of paired personal ethylbenzene exposure data for adults and children (Table 23) indicated no significant differences (Weisel et al. 2005). This confirms that indoor air in homes was the dominant exposure source for both children and adults, and that children’s characteristics and behavior did not result in greater personal exposure than what adults experienced.

Data are lacking to determine whether children of different ages receive different internal doses of ethylbenzene than adults. The only pertinent biomonitoring data located was the most recently published NHANES sampling round (2005–2006), in which quantile values for 12- to 19-year olds appeared slightly lower than those for both the total population and subjects over 60 years of age (CDC 2013). Current EPA guidance for inhalation risk assessment states that no adjustment of exposure concentrations is necessary for children other than activity weighting (US EPA 2009). Since personal concentrations account for all activities and microenvironments, the central tendency and upper-bound personal exposure estimates identified for adults are considered directly applicable to children of all ages as well (Table 24).

### Food ingestion

Ethylbenzene does not appear to be naturally occurring in plants, and bioaccumulation in the aquatic or terrestrial food chains is not expected to occur. As a result, levels in fresh (unpacked) foods are generally very low (fractions of a part per million). However, foods may be subject to accumulation of ethylbenzene by partitioning from ambient atmospheric sources, as well as migration from styrenic food packaging and contact materials. 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 (Ehret-Henry et al. 1994, Gramshaw and Vandenburg 1995, Tang et al. 2000, Nerín and Acosta 2002, Nerín et al. 2002, Wittrig 2002, Fleming-Jones and Smith 2003, Melski et al. 2003, Bradley et al. 2004, Chiesa et al. 2008, López et al. 2008, Sanagi et al. 2008).

The Total Diet Study (TDS) (or Market Basket Study) is an ongoing FDA program that determines levels of vari-
ous nutrients and chemicals, including ethylbenzene, in table-ready foods (items purchased from a supermarket and prepared for consumption as they would be in a domestic kitchen) representing the major components of the average American diet (US FDA 2004a). Taken as a whole, the TDS data indicate that ethylbenzene is a minor contributor to VOC levels in the US diet, including foods consumed in higher quantities by children. In order to conduct a more detailed evaluation of ethylbenzene intakes from the average American diet, analytical results from 1998–2001 were evaluated for the VCCEP submission (ACC 2007, Appendix M). In this updated exposure assessment, data from the 2002 FDA market basket survey were added. The most recent available data (from 2003) were not added because (1) they show very similar concentrations to those reported in 2002, and (2) changes in FDA TDS food list in 2003 complicates combination of the data sets. Mean and maximum concentrations of ethylbenzene measured in FDA market basket surveys from 1998 to 2002 were used as central tendency and upper-bound estimates of dietary concentrations (Supplementary Material, Supplement C to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Table C-2).

As shown in Figure 17, ethylbenzene concentrations have decreased in major FDA food groups since 1998. In particular, ethylbenzene was not reported in infant formulas in 2002 and 2003.

Although there are few data available concerning transfer of ethylbenzene from maternal tissues to young infants via breastfeeding, it is reasonable to believe that it would be present in human milk (e.g., Fisher et al. 1997, Needham and Wang 2002, Kim et al. 2007). In 1980, the EPA reported detection of ethylbenzene in eight samples of milk from women in five US cities (US EPA 1980). Pellizzari et al. (1982) detected ethylbenzene in eight of 12 samples of human milk collected in four urban areas. Ethylbenzene was detected in the majority of milk samples from twelve women in the Baltimore, MD metropolitan area at concentrations ranging from 0.053 to 0.58 micrograms per liter (μg/L), with a mean of 0.232 μg/L and median of 0.149 μg/L (Blount et al. 2010). In order to determine whether contamination occurred during sample collection, transportation, and storage, “collection control” aliquots of characterized milk were left exposed to room air while study participants collected their samples. Several VOCs, including ethylbenzene, were detected in these control samples, indicating the propensity for partitioning of environmental VOCs into expressed milk.

Exposure to ethylbenzene for bottle-fed children aged six months to one year was estimated based on average and maximum concentrations in milk- and soy-based infant formulas. In the Tier 1 VCCEP submission (ACC 2007, Section 6.4.1), a PBPK model was used to estimate exposure for breast-fed children up to one year of age (ACC 2007, Appendix N). Lactating women both in the general population and working in ethylbenzene production facilities were considered. The PBPK model not only included 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. The central tendency and upper-bound ethylbenzene concentrations in milk for the general public and production worker mothers, as well as in ready-to-feed soy- and milk-based infant formulas examined in the FDA’s TDS, are listed in Table 25. It is noteworthy that the concentrations of ethylbenzene in formulas are two orders of magnitude higher than those estimated in mother’s milk for the general population (both central tendency and upper-bound), around

![Figure 17. Average concentrations of ethylbenzene in selected FDA food categories, 1998–2003.](http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Table C-2)
Table 25. Central tendency and upper-bound age-weighted average ethylbenzene intakes for infants up to one year old from mother’s milk and infant formula (mg/kg-day).

| Exposure category                  | Age-weighted average ethylbenzene intakea |
|------------------------------------|------------------------------------------|
| Breast-fed (general public)        | Central tendency: $1.2 \times 10^{-5}$    |
|                                    | Upper bound: $2.6 \times 10^{-5}$        |
| Breast-fed (production worker)     | Central tendency: $2.3 \times 10^{-4}$    |
|                                    | Upper bound: $2.2 \times 10^{-3}$        |
| Bottle-fed                         | Central tendency: $3.6 \times 10^{-3}$    |
|                                    | Upper bound: $5.2 \times 10^{-3}$        |

*Calculated using modeled ethylbenzene concentrations in mother’s milk (ACC 2007, Table 6–40) and average ethylbenzene concentrations in milk- and soy-based infant formulas (Supplementary Materials, Table C-2) and EPA’s recommended age-group-specific intake rates (US EPA 2011b). Does not include other food items.

20 times higher than the central tendency estimate in milk of occupationally exposed workers, and around twice as high as the upper-bound estimate in milk of occupationally exposed workers. However, as mentioned previously, ethylbenzene was not reported in infant formulas in the 2002 and 2003 market basket surveys.

Ethylbenzene exposure assessment

Inhalation

The personal ethylbenzene inhalation exposure concentrations now available are representative of the general US population, encompassing all age groups in the urban, suburban, and rural settings distinguished in the Tier 1 VCCEP submission, and they inherently integrate individuals’ exposures in all microenvironments (Table 22). Multiplying the personal exposure concentrations for the general public in Table 22 by 0.01 provides a conservative estimate of inhalation exposure attributable to the ethylbenzene/styrene chain of commerce.

Food ingestion

Infants (0 to < 1 Year)

Ethylbenzene intake by infants less than one year old was calculated in the Tier 1 VCCEP submission using central tendency and upper-bound ethylbenzene concentrations in mother’s milk and formula, a default intake rate, and average Wt for infants of both sexes (ACC 2007, Tables 6–49 to 6–51). It was assumed that infants receive only mother’s milk or formula up to six months of age.

In the current assessment, mother’s milk and formula intake by infants less than one year old were calculated according to EPA’s currently recommended infant age groups (0 to < 1 month; 1 to < 3 months; 3 to < 6 months; and 6 to < 12 months) and milk intake rates (US EPA 2005a, 2011b), again assuming no other food up to the age of six months. Intakes by bottle-fed infants younger than six months of age were calculated by multiplying the average ethylbenzene concentration in the two listed formula types (Table 24 and Supplementary Material, Supplement C) by 0.01 provides a conservative estimate of inhalation exposure attributable to the ethylbenzene/styrene chain of commerce.

Older children and adults

In this updated exposure assessment, central tendency and upper-bound estimates of total ethylbenzene intake from the diet were calculated for prospective parents and children by combining the mean and maximum concentrations of ethylbenzene measured in FDA market basket surveys for the five-year period 1998–2002 with FDA’s age-group-specific consumption rates for ethylbenzene-containing food items (US FDA 2004b, Supplementary Material, Supplement C to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Tables C-3 and C-4). While one or more food items from each of the FDA 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. Nonetheless, because it is not known which and how many of these foods may be ingested by an individual in a given day, it was conservatively assumed that total daily intake of ethylbenzene was the sum of the intake from all food items listed.

These estimated intakes were then adjusted to comport with EPA’s age groups for exposure assessment (US EPA 2005a, 2011b) according to the following assumptions:

- Breast-fed 6 to < 12- month old = (FDA calculated intake - intake from formula + intake from mother’s milk)
- FDA age group 2 years assumed to represent both EPA 1 to < 2 years and 2 to < 3 years
- Average of FDA 2 years and 6 years assumed to represent EPA 3 to < 6 years
- Average of FDA 6 years and 10 years assumed to represent EPA 6 to < 11 years
- Average of FDA 10 years and 14–16 years assumed to represent EPA 11 to < 16 years
- Average of FDA 14–16 years and 25–30 years assumed to represent EPA 16 to < 21 years
- Average of FDA 25–30 years and 40–45 years assumed to represent EPA adult of reproductive age

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

\[
\text{Ethylbenzene intake rate}_{age} = \frac{\text{mg}}{\text{kg} \cdot \text{day}} = \sum \frac{\text{Intake}_{EB \text{in food}}}{BW_{age}} \tag{1}
\]

Where

\[
\text{Intake}_{EB \text{in food}} = \text{Age-group-specific total dietary ethylbenzene intake (Supplementary Material,}
\]
Supplement C to be found online at http://informahealthcare.com/doi/abs/10.3109/10408444.2015.1046157, Tables C-3 and C-4

BW\textsubscript{age} = Age-group-specific bwt (US EPA 2011b)

The resulting average and upper-bound estimates of total dietary intakes of ethylbenzene for each age group are presented in Figure 19. The contribution of the ethylbenzene/styrene chain of commerce can be estimated by multiplying the dietary intakes in Figure 19 by 0.25.

Summary and conclusions

As a low-molecular-weight VOC, ethylbenzene is mobile in all environmental media, with a strong tendency to migrate to the atmosphere regardless of the mode of release. Thus, the most likely route of human exposure is inhalation. Data from the two publicly available databases, NEI and TRI, document continuing decreases in ethylbenzene releases, and ambient monitoring data show declines in both outdoor and indoor concentrations over the past several decades.

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 proportionally very small. The TRI and NEI databases consistently indicate that the majority of ethylbenzene emissions are derived from mobile sources, not from major industrial point sources, and both clearly support the conclusion that industries directly involved in the ethylbenzene/styrene chain of commerce are responsible for only a very small fraction of ethylbenzene emissions in the US. Multiple air monitoring studies have shown that the primary source of ethylbenzene in indoor and outdoor air in the US is motor vehicle emissions. Thus, exposure to children and prospective parents not employed as ethylbenzene production workers is dominated by refinery chain of commerce sources such as automobile exhaust and consumer products containing mixed xylenes, and for smokers, tobacco smoke.

The approach taken in preparing the Tier 1 VCCEP ethylbenzene exposure assessment in the mid-2000s, in the absence of nationally representative indoor or personal air data, was to estimate ethylbenzene concentrations in important microenvironments (homes, schools, offices, and motor vehicles) by applying literature-based indoor/outdoor ratios to central tendency and upper-bound urban and rural outdoor air ethylbenzene concentrations. In accordance with contemporaneous EPA guidance, ethylbenzene intakes via inhalation were calculated based on assumed times spent in these microenvironments by various age groups, and default estimates of their inhalation rates and bwts. In the intervening years, multiple large studies examining indoor and personal exposures to VOCs in North America, Western Europe, and Australia have been published, and the CDC collected nationally representative personal inhalation exposure data for VOCs, including...
ethylbenzene. These data provide a more robust estimate of inhalation exposure to ethylbenzene than the previously used method by integrating exposure magnitude and duration in all areas that individuals spend time in, also accounting for individual characteristics and varying activity levels.

Current EPA guidance prescribes that potential inhalation hazards and risks are to be computed based on comparison of exposure concentrations with inhalation-based toxicity criteria (US EPA 2009). Since children do not appear to experience different personal exposures to ethylbenzene than cohabiting adults, and the guidance states that no adjustment of exposure concentrations is necessary for children other than activity weighting (US EPA 2009)—which is inherent in personal exposure data—no age-specific exposure adjustments were necessary. The updated approach presented in this paper therefore both simplifies and substantially reduces uncertainty in the ethylbenzene exposure assessment by obviating the need for (1) separate consideration of urban and rural settings, (2) time apportionment in microenvironments, and (3) assumptions regarding age-specific inhalation rates, bwts, and uptake efficiency.

Risk characterization

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 assessment, quantitative risk characterizations were done for children and prospective parents exposed to ethylbenzene.

Summary of exposure assessment

Nationally representative NHANES personal ethylbenzene inhalation exposure concentrations that inherently integrate individuals’ exposure concentrations (Jia et al. 2008c) were available for the current assessment of exposures for the general public. The similarities in paired adult–child ethylbenzene personal concentrations in the RIOPA study (Weisel et al. 2005) indicate that no adjustment is required to estimate exposures for children of all age groups (Tables 23 and 24). Ethylbenzene production workers encounter airborne levels of ethylbenzene roughly two orders of magnitude higher (on average) than those encountered by the general public (Table 23).

Intake of ethylbenzene via food ingestion was estimated for various age groups as currently recommended by EPA (US EPA 2005, 2011b). Infants younger than six months of age were assumed to consume only mother’s milk or infant formula. Dietary intakes of ethylbenzene (aged 6 months and up) were estimated from concentrations of ethylbenzene reported in FDA’s recent market basket surveys and age-group-specific consumption rates for specific food items. Intake rates for various age groups were adjusted to match the age groups recommended by EPA for exposure assessment, and normalized to EPA’s age-group-specific bwts to determine the age-specific ethylbenzene intake rates (in mg/kg/d) to be compared with the toxicity reference values (Figures 17 and 18).

One of the objectives of both the previous (ACC 2007) and current ethylbenzene exposure assessments was to semiquantitatively distinguish those proportions of each exposure pathway that are directly attributable to the ethylbenzene/styrene chain of commerce versus the petroleum chain of commerce. As discussed in the “Exposure assessment” section, the proportion of ethylbenzene in ambient air that is attributable to the ethylbenzene/styrene chain of commerce appears to be less than 0.1% based on recent relative emission estimates. Thus, use of the 1% factor estimated in the Tier 1 VCCEP submission in this updated assessment is conservative because it does
not account for the substantial reductions in ethylbenzene emissions from the ethylbenzene/styrene chain of commerce. The upper-bound estimated contribution to food items of 25% derived from kinetic modeling of potential ethylbenzene migration from food packaging in the Tier 1 submission (ACC 2007) was also applied in the current assessment.

Summary of hazard assessment

Non-cancer 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 that 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 OHC morphology in rats at concentrations of 200 ppm and greater ethylbenzene. Lifetime inhalation exposures to ethylbenzene produced pathological lesions in the mouse liver, lung, thyroid, and pituitary gland. Rats that received lifetime exposures to ethylbenzene exhibited pathological changes in 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.

A chronic non-cancer RfC of 0.3 ppm was 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) (US EPA 1991).

A chronic non-cancer RfD of 0.5 mg/kg bwt/day was proposed, 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.

Carcinogenicity

Ethylbenzene is carcinogenic in animals following lifetime exposures to high vapor concentrations. Exacerbation by ethylbenzene of CPN, a pathway that is considered to have no relevance for extrapolation to humans, is postulated as the MOA underlying the development of the rat renal cancer. Male rats that inhaled 750 ppm of ethylbenzene also appeared to have an exacerbation in testicular (LCTs) tumors, a type of tumor that occurs in nearly all aged rats of this strain. There was some evidence of liver and lung tumors in mice exposed to 750 ppm of ethylbenzene. 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 US EPA’s framework described in its Guidelines for Carcinogen Risk Assessment (US EPA 2005b), as described above.

A cancer reference value of 0.48 ppm (lower bound; central tendency = 0.80 ppm; and upper bound = 2.0 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 = 2.9 mg/kg bwt/day).

Risk assessment approaches

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

\[
HQ_{\text{inh}} = \frac{\text{Conc}}{\text{RfC}}
\]

\[
HQ_{\text{mg}} = \frac{\text{ADD}}{\text{RfD}}
\]

Where

HQ = Hazard quotient (unitless); ing = ingestion, inh = inhalation
ADD = Average daily dose (mg/kg bwt/day); and
RfD = Oral reference dose based upon non-cancer or cancer endpoints (mg/kg bwt/day).
Conc = TWA exposure concentration (ppm)
RfC = Inhalation reference concentration based upon non-cancer or cancer endpoints (ppm).

A non-linear cancer approach was considered appropriate for ethylbenzene based upon a consideration of the MOA by which tumors are produced in rodents. For this reason, the HQ approach was adopted for cancer endpoints as well as non-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.

Ethylbenzene risk characterization

The toxicity reference values were used to assess the potential non-cancer and cancer risks to children and adult populations exposed to ethylbenzene, as summarized in Tables 26, 27, and 28. Production worker exposure concentrations were adjusted to account for time spent at work (40 h/week) and time spent in other environments (128 h/week). The risk characterizations for the most highly exposed groups are discussed in greater detail below. A discussion of less highly exposed groups
would be warranted only if the HI for the most highly exposed groups exceeds acceptable levels (i.e., HI > 1).

In the xylenes VCCEP submission, modeled central tendency and upper-bound TWA xylene concentrations in indoor air for three time periods (1 h, 8 h, and 24 h) were compared to the interim Acute Exposure Guideline Level for severity level 1 effects (AEGL-1) for mixed xylenes (130 ppm). Resultant HIs ranged from 0.003 to 0.35, indicating no health concern based on xylene exposure (ACC 2005). Mixed xylenes may contain 6–15% 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 0.2–54 ppm (see “Exposure assessment” section). Dividing this range by the AEGL-1, the range of HIs would be 0.001–0.42. 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” (ACC 2005).

### Chronic risk characterization for the most highly exposed children

#### Non-cancer

The central tendency estimates for bottle-fed infants from birth to <1 month old were HQs of 0.002 for inhalation and 0.01 for ingestion, for a total HI of 0.01. The upper-bound estimates for these same infants were HQs of 0.01 for inhalation and 0.02 for ingestion, for a total HI of 0.03. These HIs indicate that even the most highly exposed children are not at risk for non-cancer effects of ethylbenzene.

#### Cancer

The central tendency estimates for bottle-fed urban infants from birth to <1 month old were HQs of 0.001 for inhalation and 0.007 for ingestion, for a total HI of 0.008. The upper-bound estimates for these same infants were HQs of 0.001 for inhalation and 0.007 for ingestion, for a total HI of 0.008. These HIs indicate that even the most highly exposed children are not at risk for lung cancer from ethylbenzene exposure.

### Risk characterization for the most highly exposed prospective parents

#### Non-cancer

The central tendency estimates for production workers who are smokers 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 0.8 for inhalation and 0.0003 for ingestion, for a total HI of 0.8. These HIs indicate that even the most highly exposed prospective parents are not at elevated risk for non-cancer effects of ethylbenzene.

Table 26. Ethylbenzene hazard characterization: HIs (HI) for adults.

| Hazard type | Assessment | Route | Adult, general public | General public, smoker | Production worker |
|-------------|------------|-------|-----------------------|------------------------|-------------------|
| Cancer      | Central Tendency | Inhalation | 0.001 | 0.002 | 0.05 |
|             |            | Oral    | 0.00009 | 0.00009 | 0.00009 |
|             |            | Total   | 0.001 | 0.002 | 0.05 |
|             | Upper Bound | Inhalation | 0.007 | 0.007 | 0.5 |
|             |            | Oral    | 0.00002 | 0.00002 | 0.0002 |
|             |            | Total   | 0.007 | 0.007 | 0.5 |
| Non-cancer  | Central Tendency | Inhalation | 0.002 | 0.003 | 0.08 |
|             |            | Oral    | 0.00001 | 0.00001 | 0.0001 |
|             |            | Total   | 0.002 | 0.003 | 0.08 |
|             | Upper Bound | Inhalation | 0.01 | 0.01 | 0.8 |
|             |            | Oral    | 0.00003 | 0.00003 | 0.0003 |
|             |            | Total   | 0.01 | 0.01 | 0.8 |

HI > 1 indicates a potential health concern.

Table 27. Ethylbenzene hazard characterization: HIs (HI) for breast-fed infants of general public and breast-fed infants of product workers.

| Hazard type | Assessment | Route | Infants of general public | Infants of product workers |
|-------------|------------|-------|----------------------------|---------------------------|
| Cancer      | Central Tendency | Inhalation | 0.001 | 0.001 | 0.001 |
|             |            | Oral    | 0.00002 | 0.00002 | 0.0002 |
|             |            | Total   | 0.001 | 0.001 | 0.001 |
|             | Upper Bound | Inhalation | 0.007 | 0.007 | 0.007 |
|             |            | Oral    | 0.00005 | 0.00005 | 0.00005 |
|             |            | Total   | 0.007 | 0.007 | 0.007 |
| Non-cancer  | Central Tendency | Inhalation | 0.0003 | 0.0003 | 0.0003 |
|             |            | Oral    | 0.00003 | 0.00003 | 0.00003 |
|             |            | Total   | 0.003 | 0.003 | 0.003 |
|             | Upper Bound | Inhalation | 0.01 | 0.01 | 0.01 |
|             |            | Oral    | 0.00008 | 0.00008 | 0.00008 |
|             |            | Total   | 0.01 | 0.01 | 0.01 |

HI > 1 indicates a potential health concern.
sources of uncertainty in the exposure assessment and toxicity assessment used in the risk characterization are discussed below.

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. 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. Since both children and adults spend most of their time in buildings, concentrations in indoor air, especially the home, are the primary determinants of exposure to the general public. Levels of ethylbenzene in indoor air are highly dependent upon and dominated by outdoor sources, but smoking and ETS likely contribute as well. In 2009, EPA issued revised guidance for inhalation exposure and risk assessment recommending that potential inhalation hazards and risks should be computed based on exposure concentrations rather than estimated intakes. Use of the nationally representative 1999–2000 NHANES study for personal exposure characterization thus obviates the need for (and eliminates the substantial uncertainties associated with) separate consideration of urban and rural settings, apportionment of time in microenvironments, and calculation of inhaled dose based on assumed inhalation rates and bwts. However, the fact that the NHANES data set represents single measurements of adult exposures taken in 1999–2000 introduces uncertainties associated with lack of child-specific data, inability to characterize individual exposure distributions, extrapolation of 48- to 72-h measurements to lifetime exposure, and probable overestimation of exposure due to documented decreases in ambient concentrations of ethylbenzene and other VOCs in the intervening years. Nonetheless, the approach taken here for comparing nationally representative personal exposures directly to relevant toxicity criteria is considered to reduce uncertainty compared with previous estimates. This approach tends to be conservative not only because of the long-term downward trend in ambient concentrations, but also because more than a quarter of NHANES subjects were likely to have elevated ethylbenzene exposures because they were smokers or had regular exposure to ETS based on serum cotinine levels.

Uncertainty in dietary intake estimates

Ethylbenzene was infrequently detected in raw and processed foods in the US, 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, that is, diffusion from the atmosphere and/or 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 with 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. Since 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

Table 28. Ethylbenzene hazard characterization: HIs (HI\(^4\)) for bottle-fed infants and children.

| Hazard type | Assessment       | Route | Birth to 1 month | 1 to <3 months | 3 to <6 months | 6 to <12 months | 1–2 years | 2–3 years | 3–6 years | 6–11 years | 11–16 years | 16–21 years |
|-------------|------------------|-------|-----------------|----------------|----------------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Cancer      | Central Tendency | Inhalation | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
|             | Oral             |       | 0.007 | 0.005 | 0.004 | 0.004 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
|             | Total            |       | 0.019 | 0.006 | 0.006 | 0.006 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Upper Bound | Inhalation       |       | 0.007 | 0.007 | 0.005 | 0.005 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
|             | Oral             |       | 0.01  | 0.008 | 0.006 | 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
|             | Total            |       | 0.02  | 0.02  | 0.01  | 0.01  | 0.009 | 0.009 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| Non-cancer  | Central Tendency | Inhalation | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
|             | Oral             |       | 0.01  | 0.01  | 0.007 | 0.006 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
|             | Total            |       | 0.02  | 0.02  | 0.01  | 0.01  | 0.008 | 0.008 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 |
|             | Oral             |       | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
|             | Total            |       | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  |

\(^4\)HI \(\geq 1\) indicates a potential health concern.
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.

Uncertainties in parameter values

Since inhalation “doses” were not calculated, the exposure parameter values used were limited to age-specific ingestion rates and bwts. The values selected were those typically used in risk assessments derived from applicable EPA and FDA guidance. These are on the conservative side, as is appropriate for this screening assessment.

Uncertainty in toxicity assessment

Study/endpoint selection

As described under “Hazard assessment” section, the toxic effects of ethylbenzene have been well studied in animals. A variety of endpoints were considered for the RfC (ototoxicity and liver effects), RfD (liver effects, hematological effects, and 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, 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. In the ACC’s initial submission for the VCCEP review (ACC 2006), mouse liver tumors were considered potentially relevant to humans, and a cancer RfD of 0.2 mg/kg/day and cancer RfC of 0.3 ppm were derived. The panel recommended that these values be eliminated from that assessment because the mouse liver tumors were deemed not relevant to human health (TERA 2007). These liver cancer values are lower than those derived in the current assessment. However, if these values were used for the risk characterization, the upper-bound HI for the most highly exposed children would still be only 0.05 and the upper-bound HI for the most highly exposed prospective parents would be 0.8. Thus, even if liver cancer were included as an endpoint potentially relevant to humans, the conclusion that the risks are acceptable would remain unchanged.

Mode of action

The MOAs by which ethylbenzene produces adverse effects in animals were assessed as described above. Since information on MOA is used to guide key decisions in the dose–response assessments (relevance to human health, dose measure, and low-dose extrapolation method), uncertainty in the MOA can have a large impact on the results.

Regarding the non-cancer 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

Since 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 is the test species due to toxicodynamic differences. However, it is equally plausible that an individual is ten times less sensitive than the average individual. However, it is equally plausible that an individual is ten times less sensitive than the average individual.

Toxicity reference value derivation

Toxicity reference values were derived using a multistep process. The point of departure was derived using BMD modeling, with internal doses calculated using PBPK models, rather than doing the dose–response analysis on the basis of external dose, and converting the external dose point of departure to an internal dose. This procedure is consistent with the observations of McLanahan et al. (2012) that use of internal dose measures can improve the agreement between the data and the BMD models. The uncertainty factors were then applied to the internal dose, and the internal RfC/RfD was converted to an external RfC/RfD using a human PBPK model, consistent with the approach used in US EPA’s methanol assessment.
of human health risk. In this assessment, thus providing a conservative assessment of steady declines (while rural concentrations remain steady), urban and suburban settings show ethylbenzene air concentrations for children and prospective parents. As ethylbenzene ingestion exceeded that from inhalation exposure and in young children (aged 1–3 years); the estimated HQ was on a par with the estimated ingestion HQ. Production workers have by far the highest exposure.

**Proposed RfC**

If BMD analysis and PBPK modeling were not used, the default ototoxicity RfC would likely be derived using one of three approaches:

1. **Otitotoxicity NOAEL = 200 ppm (audiometric threshold change)**
   - Duration adjustment = (6 h/day × 6 days/week for intermittent exposure)/(168 h/week for continuous exposure)
   - Uncertainty factors: same as above (RfC Derivation), except UFA = 10, and UFS = 10, resulting in a composite UF of 1000
   - Default ototoxicity RfC (approach 1) = 200 × (36/168)/1000 = 0.04 ppm

Alternatively, the ototoxicity RfC could be derived based on OHC3 loss as a precursor to chronic effects using the LOEL.

2. **Otitotoxicity LOEL = 200 ppm (OHC3 loss)**
   - Duration adjustment = (6 h/day × 6 days/week for intermittent exposure)/(168 h/week for continuous exposure)
   - Uncertainty factors: same as above (RfC Derivation), except UFA = 10, UFL = 3 resulting in a composite UF of 300.
   - Default ototoxicity RfC (approach 2) = 200 × (36/168)/300 = 0.1 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 (TLAEL; 95% UCL) derived by Gagnaire et al. (2007).

3. **Otitotoxicity TLAEL = 114 ppm (OHC3 loss)**
   - Duration adjustment = (6 h/day × 6 days/week for intermittent exposure)/(168 h/week for continuous exposure)
   - Uncertainty factors: same as above (RfC Derivation), except UFA = 10, resulting in a composite UF of 300.
   - Otitotoxicity RfC (approach 3) = 114 × (36/168)/300 = 0.08 ppm

Likewise, the default liver and pituitary toxicity RfCs would be derived as follows:

**Liver and Pituitary Toxicity NOAEL = 75 ppm**
   - Duration adjustment = (6 h/day × 5 days/week for intermittent exposure)/(168 h/week for continuous exposure)
   - Uncertainty factors: same as above, except UFA = 10, resulting in a composite UF of 100
   - Default liver RfC = 75 × (30/168)/100 = 0.1 ppm

The Tier 1 exposure assessment clearly demonstrated that, as expected, the dominant route of exposure to ethylbenzene is inhalation for breast-fed infants and all receptor groups aged 3 years and older. In bottle-fed infants, risk from ethylbenzene ingestion exceeded that from inhalation exposure and in young children (aged 1–3 years); the estimated HQ was on a par with the estimated ingestion HQ. Production workers have by far the highest exposure.

**Conclusions/Discussion**

**Hazard assessment**

The toxicological effects of ethylbenzene have been thoroughly studied. Overall, this information is of suitable quality to support human health hazard and risk assessments for children and prospective parents. Specialized investigations of ethylbenzene effects on hearing do indicate that 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 assessment has used a conservative interpretation of the biological significance of ototoxicity findings and hence no impact on the overall assessment is anticipated from further ethylbenzene ototoxicity investigations.

**Exposure assessment**

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.
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 (ACC 2007, Appendix O). 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. US 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 US 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. Since 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. In this assessment, a conservative effect level and uncertainty factors for this endpoint have been used to derive a potential RfC for ototoxicity that is slightly lower than that derived for 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 BMD modeling (instead of the NOAEL) to identify the point of departure, differing values for components of the composite UF (as compared with 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).

**Proposed RfD**

If BMD 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**

Default RfD = 75 × (36/168)/1000 = 0.08 mg/kg bwt/day

The existing RfD of 0.1 mg/kg bwt/day (US EPA 1991) was based on liver and kidney toxicity observed in a 182-day (sub-chronic) 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). This study was deemed unreliable (ACC 2007, Appendix O). A total uncertainty factor of 1000 (UFA = 10, UFH = 10, and UFS = 10) was applied, and a dosing schedule adjustment was made to arrive at the existing RfD. US 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 with the existing RfD, were the use of a different, chronic study, use of BMD 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 versus 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.

**Cancer reference value derivation**

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 indicates 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 that is not relevant to humans. Information regarding the MOA for lung tumors support a role for oxidative metabolites (catechols and 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 that in laboratory rodents. Furthermore, the role of oxidative stress in the formation of these tumors supports a non-linear 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 and 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.

**Risk characterization**

The risk assessment was conducted using EPA guidance. The calculated HIIs indicate that even the most highly exposed children and prospective parents are not at risk for non-cancer effects of ethylbenzene.

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**Declaration of interest**

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Supplementary materials available online
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