Appendix A: Background Information for Benzene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for benzene. The summaries are mainly based on information presented in the ATSDR (1997) toxicological profile for benzene and therefore do not represent reviews of the primary literature. The chemical structure for benzene is included in Appendix E.

A.1 Toxicokinetics

Benzene is rapidly but incompletely absorbed by humans and animals following inhalation exposure (ATSDR 1997). Results of several studies indicate that average respiratory uptake is approximately 50% in humans. For example, in a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours, absorption was 70–80% during the first 5 minutes of exposure, but subsequently decreased to about 50% (range, 20–60%) by 1 hour (Srbova et al. 1950). Respiratory uptake was approximately 47% in six subjects exposed to 52–62 ppm benzene for 4 hours (Nomiyama and Nomiyama 1974), and 52 and 48% in three subjects exposed to 1.6 or 9.4 ppm, respectively, for 4 hours (Pekari et al. 1992). In rodents, the extent of uptake increased linearly with concentration for exposures up to approximately 200 ppm (ATSDR 1997). At concentrations >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice when the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). Mice and rats have different absorption characteristics in that the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987).

Quantitative oral absorption data are not available for benzene in humans. Oral doses of benzene are extensively absorbed in animals (generally >90%), although benzene was administered in oil in many oral studies to assure predictable solubility and dose concentration control (ATSDR 1997; Cornish and Ryan 1965; Parke and Williams 1953a, 1953b; Sabourin et al. 1987). One of the studies showed that gastrointestinal absorption was >97% in rats and mice when the animals were treated with benzene by gavage at doses 0.5–150 mg/kg (Sabourin et al. 1987). Dermal absorption of benzene is low in humans and animals. Approximately 0.05% of an applied dose (0.0026 mg/cm^2) was determined in one human study (Franz 1984), and another estimated a dermal absorption rate of approximately 0.4 mg/cm^2/hour under conditions of complete saturation (35–43 cm^2 of skin was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours) (Hanke et al. 1961). Animal studies found that dermal absorption was

<1% following a single direct application of liquid benzene to Rhesus monkeys, minipigs, and hairless mice (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). Absorbed benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion rate of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (ATSDR 1997; Sato et al. 1975; Tauber 1970).

The metabolism and elimination of benzene appear to be qualitatively similar in humans and animals (ATSDR 1997; Henderson et al. 1989; Sabourin et al. 1988). Benzene is initially metabolized by cytochrome P-450-dependent mixed function oxidases primarily in the liver via several toxification and detoxification (via conjugation) pathways. Several cytochrome P-450 isozymes are involved in the metabolism of benzene, although the predominant form is CYP2E1 (Gut et al. 1993; Nakajima et al. 1993). The first metabolic step is the formation of the epoxide benzene oxide. This is followed by two toxification pathways, one involving ring hydroxylation and the second involving ring opening, resulting in the formation of putative toxic metabolites (ATSDR 1997; Henderson et al. 1989). In the first pathway involving ring hydroxylation, opening of the epoxide ring is followed by aromatization resulting in formation of phenol. Phenol is further converted into hydroquinone, which is oxidized to benzoquinone. The conjugates formed from hydroquinone (hydroquinone glucuronide and hydroquinone sulfate) are markers for this toxification pathway leading to benzoquinone. Phenol can also be metabolized to catechol and trihydroxy benzene. Metabolism of benzene oxide leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases (Henderson et al. 1989). The second pathway involving ring-opening leads to the formation of muconic acid, apparently via the precursor muconic dialdehyde.

There are two detoxification pathways. One detoxification pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated by biliary excretion (ATSDR 1997; Henderson et al. 1989; Sabourin et al. 1987; Schrenk et al. 1992). The major portion of benzene oxide is nonenzymatically rearranged to phenol. The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol. Further metabolites of phenol and benzene dihydrodiol (e.g., catechol, hydroquinone, and trihydroxy benzene) are excreted as sulfate or glucuronide conjugates and are also considered detoxification products.

Although the metabolism of benzene occurs primarily in the liver, studies in rats and mice indicate that a small amount is metabolized independently of the liver in the bone marrow, the site of characteristic benzene toxicity (Ganousis et al. 1992; Irons et al. 1980). It is believed that biotransformation is essential

for benzene-induced bone marrow damage, but it is unclear whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow (ATSDR 1997). Benzene has been found to stimulate its own metabolism (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). Differences in species, routes of exposure, and dosing regimens affect the disposition and metabolic fate of benzene (Sabourin et al. 1987, 1988, 1989a, 1989b, 1992). Benzene metabolism (both total and amounts of individual metabolites) is dose-dependent in all species studies thus far, including humans (ATSDR 1997). At low doses, more of the benzene is converted to putative toxic metabolites than at high doses. At high doses, benzene inhibits phenol metabolism to hydroquinone, apparently through competition for a common site on the CYP2E1 isozyme to which hydroquinone and catechol also bond. The effect that dose and species can have on the metabolism of benzene is illustrated by a study in which rats and mice were administered benzene by gavage at doses of 0.5–150 mg/kg (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of the administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. Total metabolites did not increase at higher doses in mice, suggesting saturation of metabolic pathways. Other studies similarly indicate that mice metabolize benzene more efficiently than rats (Medinsky et al. 1989a, 1989b; Travis et al. 1990).

Human and animal data show that exhalation is the main route for elimination of unmetabolized benzene, metabolites are excreted predominantly in the urine, and only a small amount of the absorbed amount is eliminated in feces (ATSDR 1997). Respiratory uptake (the amount of benzene absorbed from the lungs) and respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 47 and 17%, respectively, in six humans exposed to 52–62 ppm benzene for 4 hours (Nomiyama and Nomiyama 1974). Results from a study of 23 humans who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6 and 0.07–0.2% of the retained benzene was excreted in the breath (within 5–7 hours) and urine, respectively (Srbova et al. 1950). The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor concentrations of 10–1,000 ppm, the mice received 150–200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5 and <9% of the radioactivity in the rats and mice, respectively. At lower exposure concentrations (11–130 ppm), both rats and mice excreted <6% of the radioactivity in the expired air. At the highest exposure concentrations (870 ppm in rats, 990 ppm in mice), both rats and mice exhaled a significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of exposure. The percentage of total excreted radioactivity that was

not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. Approximately 90% of the radioactivity was excreted as urinary metabolites by rats at ≤260 ppm and by mice at ≤130 ppm. The total urinary metabolite formation was 5–37% higher in the mice than in the rats at all doses, apparently due to the greater amount of benzene inhaled by mice per kg of body weight. The effect of dose on excretion of radioactivity was also studied in rats and mice following oral administration of 0.5–300 mg/kg single doses of ^{14}C-benzene (Sabourin et al. 1987). At doses <15 mg/kg, ≥90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of radioactivity in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. Of a 150 mg/kg dose, 50 and 69% was eliminated in the expired air largely as unmetabolized benzene in rats and mice, respectively.

Four PBPK models have been developed to describe the behavior of benzene in rats, mice, and humans. The Medinsky model addresses species differences in benzene kinetics using rats and mice (Medinsky et al. 1989a, 1989b, 1989c). The Travis model specifically addresses human pharmacokinetics of benzene in comparison to experimental animal data (Travis et al. 1990), whereas the Bois and Paxman model (Bois and Paxman 1992) addresses the effect of exposure rate on benzene metabolism. The Sun model (Sun et al. 1990) addresses the formation of hemoglobin-benzene derived adducts in the blood, as a tool in monitoring benzene exposure.

A.2 Health Effects

The nervous and hematopoietic systems are the main targets of benzene. Acute inhalation or oral exposure to high levels of benzene has caused symptoms and signs of central nervous system toxicity in humans and animals (ATSDR 1997; Carpenter et al. 1944; Cornish and Ryan 1965; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Effects such as dizziness, vertigo, tremors, narcosis, and cardiac arrhythmias have been observed following both acute nonlethal and lethal exposures. Intermediate-duration inhalation and oral exposures to benzene induced neurological effects in animals that included reduced limb grip strength, behavioral disturbances, and changes in brain levels of monoamine transmitters and acetylcholinesterase (Dempster et al. 1984; Frantik et al. 1994; Hsieh et al. 1988; Li et al. 1992).

Hematotoxicity is the most noted and characteristic systemic effect resulting from intermediate and chronic benzene exposure in humans and animals. All of the major types of blood cells are susceptible

(erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A common clinical finding is cytopenia, which is a decrease in various cellular elements manifested as anemia, leukopenia, or thrombocytopenia in humans (ATSDR 1997). Benzene-associated cytopenias vary and can involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood. Prolonged exposure to benzene can cause severe damage to the bone marrow involving cellular aplasia in humans and animals. This condition, known as aplastic anemia, is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow (ATSDR 1997). Benzene-induced aplastic anemia can progress to AML, which is characterized by the appearance in the peripheral blood of cells morphologically indistinguishable from myeloblasts (Aksoy 1980; Aksoy et al. 1974; Doskin 1971; Rozen et al. 1984). Human studies that provide some estimate of levels of exposure indicate that adverse hematological effects occurred at levels >10 ppm and generally not at levels <1 ppm (ATSDR 1997). Adverse hematological effects begin to appear in animals at benzene concentrations of 10–100 ppm and above. Oral data are essentially limited to findings in intermediate- and chronic-duration animal studies showing that loss of blood elements occurs following exposure to benzene in drinking water or by gavage at doses as low as 8–25 mg/kg/day (ATSDR 1997).

Other health effects of benzene include immunological changes in humans and animals, which appear to be largely related to decreases in circulating leukocytes and the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies (ATSDR 1997). Adequate reproductive and developmental toxicity data on benzene are essentially limited to results of inhalation studies in animals (ATSDR 1997). There is suggestive evidence of benzene-induced testicular effects (e.g., atrophy/ degeneration, decrease in spermatozoa, increase in abnormal sperm forms), particularly in mice following intermediate duration exposure to 300 ppm (ATSDR 1997; Ward et al. 1985; Wolf et al. 1956). Results of developmental toxicity studies indicate that inhalation exposure to high levels of benzene is fetotoxic and maternally toxic in several species as shown by decreased fetal weight and/or minor skeletal variants. Fetotoxic effects in rodents occurred at benzene levels ≥47 ppm (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985), although there was evidence of transient hematopoietic anomalies in fetuses and offspring of mice exposed to 5–20 ppm benzene (Keller and Snyder 1986, 1988). Chromosomal damage in bone marrow cells and lymphocytes are well documented effects of benzene based on findings in human occupational studies and *in vivo* studies in animals (ATSDR 1997). As indicated above, there is a well established causal relationship between occupational exposure to benzene and acute myelogenous leukemia (ATSDR 1997; Aksoy et al. 1971, 1972, 1974). Benzene-induced leukemia has not been clearly demonstrated in laboratory animals, although increased incidences of lymphomas, Zymbal gland carcinomas, and other

neoplasms were found in rats and mice following chronic inhalation or oral exposure (ATSDR 1997; Cronkite et al. 1984, 1985, 1986, 1989; Farris et al. 1993; NTP 1986; Snyder et al. 1980, 1984, 1988).

A.3 Mechanisms of Action

The most characteristic toxic effect of benzene is a decrease in bone marrow cellularity, which appears to ultimately lead to aplastic anemia and development of leukemia. The compensatory proliferative response (regenerative hyperplasia) to the anemia observed in the bone marrow, thymus, and spleen of exposed animals may play a role in the carcinogenic response (Rozen and Snyder 1985; Snyder 1987; Snyder and Koscis 1975; Snyder et al. 1984, 1993; Toft et al. 1982).

Metabolites appear to play key roles in the development of the hematotoxic, clastogenic, and carcinogenic effects of benzene. Studies of benzene metabolism and mechanisms of toxic action have identified or postulated a number of biologically reactive metabolites, including benzene oxide, benzene dihydrodiol, hydroquinone, catechol, benzoquinones, and muconaldehyde (ATSDR 1997). It is generally believed that benzene metabolites covalently bind to cellular macromolecules (including DNA, ribonucleic acid [RNA], and proteins), thereby leading to dysfunction in the bone marrow (including stem, progenitor, and stromal cells) and other tissues. For example, benzene and its metabolites may induce oxidative DNA damage or interfere with the incorporation of iron into bone marrow precursors (Longacre et al. 1981; Snyder and Kalf 1994; Snyder et al. 1989), chelates of iron and hydroquinone or 1,2,4-benzenetriol appear to be potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and hydroquinone may accumulate in bone marrow to act as substrates for myeloperoxidase, forming benzoquinone which is myelotoxic and clastogenic (ATSDR 1997; Medinsky et al. 1994).

The relationship between adduct formation and toxicity is not clear, and multiple metabolic pathways and mechanisms are involved in benzene toxicity and carcinogenicity. The multiple metabolic pathways provide opportunities for modulation of benzene metabolism, either by competition with other chemicals for the available cytochrome P-450 sites, by induction or inhibition of the oxidation or conjugation enzymes, or by direct competition between benzene and its metabolites (Medinsky et al. 1994). There also may be synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or between glutathione-depleting metabolites of benzene and oxygen radicals (generated by futile cycling of cytochrome P-450 or cycling of quinone metabolites) (Parke 1989).

The acute neurological effects of benzene are similar to the general anesthetic effects of other lipophilic solvents, and consequently are presumed to result from a direct effect of the parent compound on central nervous system cell membranes unrelated to its metabolites (Snyder et al. 1993). There is a paucity of mechanistic information on the chronic nervous system toxicity of benzene (ATSDR 1997; Snyder et al. 1993).

A.4 Health Guidelines

ATSDR (1997) derived an acute-duration inhalation MRL of 0.05 ppm for benzene based on a LOAEL of 10 ppm for immunotoxicity (reduced lymphocyte proliferation) following mitogen stimulation in mice (Rozen et al. 1984). The animal LOAEL was converted to a human equivalent concentration ($LOAEL_{HEC}$) of 14.7 ppm and divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) to yield the MRL. The mice were exposed 6 hours/day for 6 days.

ATSDR (1997) derived an intermediate-duration inhalation MRL of 0.004 ppm for benzene based on a LOAEL of 0.78 ppm for neurological effects (increased rapid response time) in mice (Li et al. 1992). The animal LOAEL was converted to a $LOAEL_{HEC}$ of 0.33 ppm and divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) to yield the MRL. The mice were exposed 6 hours/day, 6 days/week for 30 days.

ATSDR (1997) did not derive a chronic-duration inhalation MRL or acute-, intermediate-, or chronic-duration oral MRLs for benzene due to lack of suitable data.

The EPA Integrated Risk Information System (IRIS) database does not list a RfD or RfC for benzene (IRIS 2001). EPA has classified benzene as a "known" human carcinogen (Category A) under the 1986 Risk Assessment Guidelines (IRIS 2001). Under EPA's proposed revised Carcinogen Risk Assessment Guidelines, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2001). Based on leukemia data in humans, an inhalation unit risk range of $2.2x10^{-6}$–$7.8x10^{-6}$ per $\mu g/m^3$ was estimated for benzene (IRIS 2001). The inhalation unit risk range was extrapolated to an oral (drinking water) unit risk range of $4.4x10^{-4}$–$1.6x10^{-3}$ per mg/L (IRIS 2001).

The NTP (2001) concluded that benzene is known to be a human carcinogen. IARC (1987) classified benzene as a Group 1 carcinogen (carcinogenic to humans).

ACGIH (2001) recommends a TLV-TWA of 0.5 ppm and short-term exposure limit/ceiling (STEL/C) of 2.5 ppm for benzene based on cancer as the critical effect. ACGIH has included benzene in the carcinogenicity category A1 (confirmed human carcinogen).

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Appendix B: Background Information for Toluene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for toluene. The summaries are mainly based on information presented in the ATSDR (2000) toxicological profile for toluene and therefore do not represent reviews of the primary literature. The chemical structure for toluene is included in Appendix E.

B.1 Toxicokinetics

Studies with humans and animals indicate that toluene is readily absorbed from the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin (ATSDR 2000). Respiratory uptake was rapid in humans exposed to 80 ppm toluene, as shown by the appearance of toluene in the blood within 10–15 minutes of exposure (Hjelm et al. 1988). About 50% of deuterium-labeled toluene was absorbed from the lungs in volunteers exposed to 53 ppm for 2 hours during a period of light exercise (Lof et al. 1993). Seven humans exposed to 50 ppm toluene in a closed chamber showed an average retention of 83% of the inspired concentration (Benoit et al. 1985). Complete gastrointestinal absorption in humans was indicated by monitoring exhaled air for toluene and urine for toluene metabolites (hippuric acid and *ortho*-cresol) following oral administration of toluene as a 2 mg/minute infusion for 3 hours through a feeding tube into the stomach (Baelum et al. 1993). Complete absorption of orally administered toluene has also been observed in rats, although the rate of oral absorption was slower than pulmonary absorption (maximum blood levels were observed 1.5–3 hours and 15–30 minutes after administration, respectively) (Pyykko et al. 1977). The rate of dermal absorption of liquid toluene through human forearm skin was found to be slow, ranging from 14 to 23 mg/cm^2/hour (Dutkiewicz and Tyras 1968). Dermal absorption of toluene vapor has been demonstrated in animals (ATSDR 2000). For example, dose and duration-related increases in whole body toluene levels were found in nude mice that were exposed to 300, 1,000, or 3,000 ppm toluene under conditions where there was no respiratory intake of toluene (Tsuruta 1989). The calculated skin absorption coefficient was 1.24 cm/hour. The skin absorption rates were 0.0009, 0.0046, and 0.0144 mg/cm^2/hour for the 300, 1,000 and 3,000 ppm concentrations, respectively.

Distribution of absorbed toluene in humans and laboratory animals is characterized by preferential uptake in lipid-rich and highly vascular tissues such as the brain, bone marrow, and body fat (ATSDR 2000). Toluene is distributed between the plasma and red blood cells at approximately a 1:1 ratio in humans based on *in vitro* data and a 1:2 ratio in rats based on *in vivo* data (Lam et al. 1990). In the red blood cells, toluene appears to be associated with the hemoglobin rather than the cell membrane. The

interaction of toluene with the red blood cell increases the amount of toluene that can be accommodated by the aqueous blood medium and facilitates transport of toluene to all areas of the body (including the brain) at a rate that is greater than if toluene was transported only in the plasma. Within the brain, toluene has a greater affinity for the areas that contain lipid-rich white matter, such as the brain stem and midbrain, rather than the areas with larger amounts of gray matter (Ameno et al. 1992; Bergman 1979).

The first step in toluene metabolism is catalyzed by several cytochrome P-450 species (ATSDR 2000; Gut et al. 1993; Nakajima and Wang 1994; Nakajima et al. 1991, 1992a, 1992b, 1993, 1997; Tassaneeyakul et al. 1996). Most toluene is initially metabolized by side-chain hydroxylation to form benzyl alcohol, followed by oxidation to benzoic acid. The main CYP isozyme involved in the formation of benzyl alcohol is CYP2E1, although CYP2B1, CYP2B6, CYP2C6, CYP1A2, and CYP1A1 (in decreasing order) are also active. Most of the benzoic acid is then conjugated with glycine to form hippuric acid, although some can be conjugated with uridine-5'-diphosphate (UDP)-glucuronate to form the acyl-glucuronide. In both humans and rats, up to about 75–80% of inhaled toluene that is absorbed can be accounted for as hippuric acid in the urine. Much of the remaining toluene is exhaled unchanged. A very small portion (<1–5%) of absorbed toluene undergoes ring hydroxylation by CYP1A2, CYP2B2, or CYP2E1 to form 2,3- and 3,4-epoxide intermediates and subsequently *ortho*- or *para*-cresols, which are conjugated with sulfate or glucuronate (Baelum et al. 1993, Nakajima and Wang 1994; Nakajima et al. 1997; Tassaneeyakul et al. 1996). Other minor metabolites include S-benzyl mercapturic acid and S-*p*-toluylmercapturic acid, which are thought to be formed by a series of steps beginning with benzyl alcohol and 3,4-toluene expoxide, respectively (Angerer et al. 1998). The liver is expected to be the main site of toluene metabolism, based on the concentration of CYP isozymes in the liver relative to other tissues. Studies in rats indicate that the expression of hepatic CYP isozymes is influenced by various factors, including age, sex, and level of toluene exposure (Nakajima and Wang 1994).

As indicated above, studies with humans and laboratory animals indicate that following acute periods of inhalation exposure to toluene, absorbed toluene is excreted predominately in the urine as metabolites and, to a lesser extent, as nonmetabolized toluene in exhaled air (Lof et al. 1993; Ogata 1984; Tardif et al. 1998). For example, following a 2-hour exposure with light physical exercise to deuterium-labeled toluene at a concentration of 200 mg/m^3 (53 ppm), an average 78% of retained label was excreted as urinary hippuric acid within 20 hours by a group of nine volunteers (Lof et al. 1993). A significant portion of absorbed toluene in this and other studies has been estimated to be exhaled as nonmetabolized toluene (7–20% of absorbed toluene) (Carlsson 1982; Leung and Paustenbach 1988; Lof et al. 1993). Analyses of kinetic data for toluene concentrations in blood, exhaled breath, or adipose tissue following

inhalation exposure of humans and rats indicate that most absorbed toluene is rapidly eliminated from the body and that a smaller portion (that which gets into adipose tissues) is slowly eliminated (Leung and Paustenbach 1988; Lof et al. 1993; Pellizzari et al. 1992; Pierce et al. 1996, 1999; Rees et al. 1985). For example, using PBPK models, mean terminal half-lives of about 30–38 hours were calculated for changes in blood toluene concentrations between 50 and 100 hours after cessation of 2-hour inhalation exposures of male subjects to 50 ppm $^{1}H_{8}$-toluene and 50 ppm $^{2}H_{8}$-toluene (Pierce et al. 1996, 1999). During this terminal phase of disposition, >95% of toluene is expected to be in adipose tissue and the release of toluene from adipose tissues has been proposed to be the rate-limiting step (Pierce et al. 1999). Elimination half-lives ranged from about 12 to 65 hours in subcutaneous adipose tissue samples taken from 12 subjects at several times within 8 days of cessation of exposure to about 80 ppm toluene for four consecutive 30-minute periods, and increasing elimination half-lives were significantly correlated with increasing amounts of body fat (Carlsson and Ljungquist 1982).

PBPK models are available that describe the kinetics of toluene after inhalation exposure in humans (Fisher et al. 1997; Lapare et al. 1993; Pierce et al. 1996, 1999) and rats (DeJongh and Blaauboer 1996, 1997; Tardif et al. 1993b).

B.2 Health Effects

The nervous system is the critical target of toluene toxicity following acute, intermediate, or chronic inhalation or oral exposure to toluene (ATSDR 2000). Effects on the human nervous system from inhaled toluene are well documented. Studies with volunteers under controlled acute (6–8 hours) exposure conditions indicate that subtle neurological impairment is detectable in most subjects at concentrations in the 75–150 ppm range (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991; Guzelian et al. 1988; Iregren 1986; Rahill et al. 1996). Concentrations of 200–800 ppm can produce exhilaration and light-headedness, and, at higher acute exposure concentrations, intellectual, psychomotor, and neuromuscular abilities are obviously impaired followed by development of narcosis (EPA 1985; von Oettingen et al. 1942). Numerous case studies have associated chronic inhalation exposure to toluene at levels inducing narcosis and euphoria (estimated 4,000–12,000 ppm) with residual or permanent neurological damage as evidenced by abnormal electroencephalograms, structural changes in the brain, tremors, paranoid psychosis, recurrent hallucinations, and impaired speech, hearing, and vision (ATSDR 2000). Studies of workers repeatedly exposed to toluene in workplace air at concentrations ranging from about 30 to 150 ppm have found evidence for increased incidence of self-reported neurological symptoms, performance deficits in neurobehavioral tests, hearing loss, changes in visual-evoked

brainstem potentials, and color vision impairment (Abbate et al. 1993; Boey et al. 1997; Foo et al. 1990; Morata et al. 1997; Orbaek and Nise 1989; Vrca et al. 1995, 1997a, 1997b; Yin et al. 1987; Zavalic et al. 1998a, 1998b, 1998c).

Supporting neurological effects data come from inhalation studies of toluene-exposed animals showing changes in behavior, hearing loss, and subtle changes in brain structure, electrophysiology, and levels of neurotransmitters (ATSDR 2000). For example, performance deficits in trained neuromuscular responses occurred in rats exposed concentrations as low as 125 ppm toluene for 4 hours (Kishi et al. 1988; Mullin and Krivanek 1982; Wood et al. 1983), changes in brain biochemical variables (e.g., dopamine levels, dopamine D2 receptor binding, changes in glial fibrillary acidic protein) were induced in rats exposed to concentrations as low as 50–80 ppm for 6–8 hours/day (API 1997; Hillefors-Bergllund et al. 1995; Ikeda et al. 1986; Little et al. 1998; von Euler et al. 1989, 1993, 1994), hearing loss occurred in rats exposed to concentrations as low as 700–1,000 ppm, 6–14 hours/day for 2–9 weeks (Campo et al. 1997, 1998; Johnson et al. 1988; Pryor and Rebert 1992; Pryor et al. 1984a, 1984b, 1991), and decreases in brain weight and phospholipid content occurred in rats continuously exposed to 320 ppm for 30 days (Kyrklund et al. 1987). Neurological effects observed in animals after acute- or intermediate-duration oral exposure include changed flash-evoked potentials in rats given single gavage doses of toluene as low as 250 mg/kg (Dyer et al. 1988), and changes in brain levels of several neurotransmitters (e.g., norepinephrine, dopamine, serotonin) in mice exposed to 5–105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1990).

Toluene has caused respiratory tract irritation in humans following acute-duration inhalation exposure to concentrations above approximately 100 ppm, but there is little evidence for other adverse effects (ATSDR 2000). Studies of chronic toluene abusers or occupationally exposed humans have provided little evidence for serious liver damage due to inhaled toluene. Increases in liver weight (likely associated with microsomal enzyme induction), but no significant hepatic histological changes, were found in rats and mice following acute, intermediate, or chronic exposure to concentrations above 300 ppm (ATSDR 2000). The kidney may be a target of toluene toxicity following exposure to very high levels of toluene. Renal acidosis was observed in solvent abusers exposed to toluene, but the renal dysfunction usually reversed when exposure ceased, and the cases were frequently confounded by probable exposure to multiple solvents (ATSDR 2000). Kidney damage (e.g., renal tubular casts) was induced in rats after intermediate- and chronic-duration exposure to concentrations above 600 ppm (CIIT 1980; NTP 1990).

There is suggestive evidence that toluene may cause some reproductive problems, especially with repeated inhalation exposure during pregnancy to concentrations above 200 ppm (ATSDR 2000). Increased risks of spontaneous abortions were observed in women occupationally exposed to toluene, or wives of men similarly exposed (Lindbohm et al. 1992; Ng et al. 1992; Taskinen et al. 1989). However, interpretation of these results is limited due to small sample size evaluated, an inability to define accurate exposure levels, failure to account for all possible confounding variables, and the difficulty in validating self-reported data. Occupational exposure to increasing concentrations of toluene (8–111 ppm) has been associated with decreased plasma levels of the luteinizing hormone, follicle stimulating hormone and testosterone levels in males (Svensson et al. 1992a, 1992b). Studies in animals found some minor toluene-induced changes in male and female reproductive organs (e.g., decreased sperm count in male rats [Ono et al. 1995, 1996] and ultrastructural changes in antral follicles in ovary of female rats [Tap et al. 1996]), but no histological evidence of structural damage to the reproductive organs in rats and mice exposed orally for intermediate durations or by inhalation for intermediate or chronic durations (NTP 1990). No evidence for impaired reproductive performance was found in several assays (Ono et al. 1995, 1996; Smith 1983; Thiel and Chahoud 1997), including a 2-generation study of rats intermittently exposed to up to 2,000 ppm (API 1985), although gestational exposure to toluene caused increased fetal mortality in rats intermittently exposed to 2,000 ppm (Ono et al. 1995, 1996), and increased abortions in rabbits continuously exposed to 267 ppm but not 133 ppm (Ungvary and Tatrai 1985).

A number of reports of birth defects in children born to women who abused toluene or other organic solvents during pregnancy suggest that high-level (4,000–12,000 ppm) exposure to toluene during pregnancy can be toxic to the developing fetus, causing effects that included microcephaly, central nervous system dysfunction, growth deficiency, cranofacial and limb abnormalities, and reversible renal tubular acidosis (ATSDR 2000). Results from several inhalation exposure studies of animals indicate that exposure to levels of toluene that begin to produce maternal toxicity can cause fetal effects, including reduced fetal survival and retardation of growth and skeletal development (Courtney et al. 1986; Hudak and Ungvary 1978; Huntingdon Research Centre 1992a, 1992b; Ono et al. 1995; Thiel and Chahoud 1997; Ungvary and Tatrai 1985). No-effect levels in animals for toluene effects on standard developmental endpoints ranged from about 133–750 ppm. In animal studies of oral exposure during gestation, no developmental effects were observed in pregnant mice exposed to doses of ≥1,800 mg/kg/day (Seidenberg et al. 1986; Smith 1983), but exposure of pregnant rats to gavage doses of 650 mg/kg/day produced offspring with decreased body weights, delayed ossification, smaller brain volumes, and decreased forebrain myelination per cell compared with controls (Gospe and Zhou 1998; Gospe et al. 1996). Results from studies of neurobehavioral endpoints in rats following gestational

exposure to toluene suggest that intermittent inhalation exposure to concentrations >1,200 ppm can impair offspring behavioral development (Jones and Balster 1997; Ono et al. 1995; Thiel and Chahoud 1997) and that drinking water exposure during gestation and lactation at doses of 106 mg/kg/day changes postweaning open-field locomotor activity in rat offspring (Kostas and Hotchin 1981).

Human and animal studies generally do not support a concern for the carcinogenicity of toluene. Eleven human epidemiology studies assessed toluene exposure as a possible risk factor for cancer (ATSDR 2000). Cancers of most sites were not significantly associated with toluene exposure in any study and there was weak consistency in the findings of those studies that did find association of a particular cancer type with toluene exposure. Three cohort studies involved workers occupationally exposed predominantly to toluene, whereas the remainder of the human studies primarily involved subjects exposed to mixtures of solvents including toluene (Antilla et al. 1998; ATSDR 2000; Svensson et al. 1990; Walker et al. 1993). The information from the human studies is inadequate to assess the carcinogenic potential of toluene, mainly because of the lack of consistent findings across the studies and the likelihood that many of the studied groups were exposed to multiple chemicals. Chronic bioassays in animals found no dose-related increased incidences of neoplastic lesions in rats or mice exposed by inhalation or in orally exposed rats (CIIT 1980; Maltoni et al. 1997; NTP 1990). Toluene was generally nongenotoxic in *in vivo* studies of exposed humans, *in vitro* microbial assays, and other *in vitro* test systems (ATSDR 2000).

B.3 Mechanisms of Action

The mechanism by which acute exposure to toluene brings about neurological effects such as central nervous system depression and narcosis is generally thought to involve, at least in part, reversible interactions between toluene (the parent compound and not its metabolites) and components (lipids or proteins) of nervous system membranes (ATSDR 2000). Support of parent-material involvement comes from the observation that pretreatment of rats with phenobarbital increased the rate of *in vivo* toluene metabolism and shortened the time of recovery from narcosis from single intraperitoneal doses of toluene (Ikeda and Ohtsuji 1971). Other support for this hypothesis includes the transient nature of anesthesia from acute high level exposure to toluene and the rapidity with which toluene-induced changes in brain biochemical variables can be measured (Korpela and Tahti 1988; Lebel and Schatz 1988, 1989, 1990; Rea et al. 1984). On a molecular level, the acute anaesthetic actions of toluene and other agents have been postulated to involve intercalation of toluene into the lipid bilayer of nerve membranes and/or reversible interactions with proteins in the membrane (Franks and Lieb 1985, 1987).

Clinically obvious neurological impairment (e.g., gait and speech abnormalities) and brain atrophy have been observed in several cases of chronic toluene-inhalation abuse. Magnetic resonance imagery (MRI) of the brain of solvent abusers (Filley et al. 1990; Rosenberg et al. 1988a, 1988b) suggest preferential atrophy in lipid-rich regions of the brain (ATSDR 2000). The MRI changes may be related to lipid compositional changes in the white matter, since these regions are more lipid-rich than gray matter (Ameno et al. 1992). The observations are consistent with a hypothesis that chronic exposure to high concentrations of toluene brings about structural changes in the brain related to lipid compositional changes (ATSDR 2000). Supporting evidence for this hypothesis includes altered phospholipid composition of brain synaptosomes, decreased phospholipid concentrations in the cerebral cortex, and decreased number of neurons in the hippocampus in toluene-exposed rats (Korbo et al. 1996; Kyrklund et al. 1987; Lebel and Schatz 1988, 1989, 1990). It is uncertain if toluene-induced changes in membrane phospholipid content may be caused by increased breakdown of phospholipids or inhibition of synthesis.

B.4 Health Guidelines

ATSDR (2000) derived an acute-duration inhalation MRL of 1 ppm for toluene based on a NOAEL of 40 ppm for subjective neurological effects (headaches, dizziness, and feelings of intoxication) and eye and nose irritation in humans (Andersen et al. 1983) and an uncertainty factor of 10 (to account for human variability). Volunteers were experimentally exposed to toluene 6 hours/day for 4 consecutive days.

ATSDR (2000) did not derive an intermediate-duration inhalation MRL for toluene due to lack of suitable data, but the chronic inhalation MRL would also be protective for intermediate-duration exposures.

ATSDR (2000) derived a chronic-duration inhalation MRL of 0.08 ppm for toluene based on a LOAEL of 35 ppm for neurological effects (color vision impairment) in humans (Zavalic et al. 1998b) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). Workers were occupationally exposed to benzene for an average of 16 years.

ATSDR (2000) derived an acute-duration oral MRL of 0.8 mg/kg for toluene based on a LOAEL of 250 mg/kg/day for neurological effects (decreased flash-evoked potential) in rats (Dyer et al. 1988) and an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were administered a single dose of toluene by gavage.

ATSDR (2000) derived an intermediate-duration oral MRL of 0.02 mg/kg/day for toluene based on a LOAEL of 5 mg/kg/day for neurological effects (regional brain increases in monoamine neurotransmitters) in mice (Hsieh et al. 1990) and an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were exposed to toluene in drinking water for 28 days.

ATSDR (2000) did not derive a chronic-duration oral MRL for toluene due to lack of suitable data.

The EPA IRIS database lists an RfD and an RfC for toluene (IRIS 2001). The RfD is based on a NOAEL of 312 mg/kg/day (converted to 223 mg/kg/day) for changes in liver and kidney weights in rats (NTP 1990) and an uncertainty factor of 1,000 (applied to account for inter- and intraspecies extrapolations, for subchronic-to-chronic extrapolation, and for limited reproductive and developmental toxicity data). The animals were exposed to toluene in corn oil by gavage 5 days/week for 13 weeks.

The EPA RfC for toluene is based on a LOAEL of 88 for neurological effects in humans (Foo et al. 1990) and an uncertainty factor of 300 (10 for use of a LOAEL, 10 for human variability, and 3 for database deficiencies) (IRIS 2001). Workers were occupationally exposed to toluene for an average of 5.7 years.

ACGIH (2001) recommends a TLV-TWA of 50 ppm for toluene based on central nervous system effects (reports of headache and irritation in humans associated with 4–6 hours of continuous inhalation of toluene).

The NTP (2001) has not listed toluene as a known or anticipated human carcinogen. EPA determined that toluene is not classifiable as to carcinogenicity (Category D) based on no human data and inadequate animal data (IRIS 2001). IARC (1999a) concluded that toluene is not classifiable as to its carcinogenicity (Group 3) to humans based on inadequate evidence in humans and evidence suggesting lack of carcinogenicity in animals.

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Appendix C: Background Information for Ethylbenzene

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for ethylbenzene. The summaries are mainly based on information presented in the ATSDR (1999b) toxicological profile for ethylbenzene and therefore do not represent reviews of the primary literature. The chemical structure for ethylbenzene is included in Appendix E.

C.1 Toxicokinetics

Ethylbenzene is well absorbed in humans via the inhalation and dermal routes of exposure, although oral absorption data in humans are lacking (ATSDR 1999b). For example, volunteers exposed for 8 hours to ethylbenzene at concentrations of 23–85 ppm were shown to retain 64% of the inspired vapor, with only trace amounts detected in expired air (Bardodej and Bardodejova 1970). Another inhalation study that involved humans exposed to similar levels of ethylbenzene demonstrated mean retention rates of 49%, suggesting possible variability of absorption rates among individuals (Gromiec and Piotrowski 1984). Dermal absorption rates of 24–33 and 0.11–0.23 mg/cm^2/hour have been measured for male subjects exposed to liquid ethylbenzene and ethylbenzene from aqueous solutions, respectively (Dutkiewicz and Tyras 1967). The average amounts of ethylbenzene absorbed after volunteers immersed one hand for up to 2 hours in an aqueous solution of 112 or 156 mg/L ethylbenzene were 39.2 and 70.7 mg ethylbenzene, respectively. Animal data support the human inhalation and dermal findings and further indicate that absorption rates are high following oral exposure as well, as indicated by 72–92% urinary recovery of single oral doses in rats and rabbits (Climie et al. 1983; El Masri et al. 1956).

Information on the distribution of ethylbenzene in humans is available from one inhalation study indicating rapid distribution to adipose tissues throughout the body (Engstrom and Bjurstrom 1978). Oral and inhalation studies in animals support these results (Chin et al. 1980a, 1980b; Climie et al.1983). Ethylbenzene is accumulated primarily in the liver, kidney, and fat. In rats, the concentrations of ethylbenzene in perirenal adipose tissue were reported to increase, although not linearly, with increasing concentrations of ethylbenzene and in a mixture of solvent vapors containing ethylbenzene (Elovaara et al. 1982; Engstrom et al. 1985). The less-than-linear increase of ethylbenzene in adipose tissue with increasing dose was partially attributed to the induction of drug-metabolizing enzymes occurring with increasing exposure concentrations, altered blood flow to adipose tissue, changes in lung excretion, and changes in the distribution of ethylbenzene in different tissues.

Ethylbenzene is metabolized in humans mainly through hepatic cytochrome P-450-mediated side chain oxidation (hydroxylation) to initially form 1-phenylethanol, from which several metabolites are produced that are excreted in the urine (ATSDR 1999b). Isozymes involved in the initial oxidation include CYP2E1 and CYP1A2 (Gut et al. 1993). The major urinary metabolites of ethylbenzene in humans exposed via inhalation are mandelic acid (approximately 64–71%) and phenylglyoxylic acid (approximately 19–25%) (Bardodej and Bardodejova 1970; Engstrom et al. 1984). Minor pathways (e.g., ring hydroxylation) in humans yield hydroxylated derivatives (e.g., *p*-hydroxyacetophenone, *m*-hydroxy-acetophenone, 1-phenyl-1,2-ethanediol, acetophenone, 2-hydroxyacetophenone, and 4-ethylphenol) that are conjugated with glucuronide or sulfate. The principal metabolic pathway in rats is believed to begin with hydroxylation of the side chain as in humans, although the major urinary metabolites following inhalation or oral exposure were identified as hippuric and benzoic acids (approximately 38%), 1-phenyl-ethanol (approximately 25%), and mandelic acid (approximately 15–23%), with phenylglyoxylic acid making up only 10% of the metabolites (Climie et al. 1983; Engstrom 1984; Engstrom et al. 1985). Both *in vivo* studies using rats and *in vitro* studies using rat liver microsomes showed that 4-ethylphenol was also produced from ethylbenzene, perhaps by rearrangement of corresponding arene oxides (Bakke and Scheline 1970; Kaubisch et al. 1972). In rabbits, the most important metabolite is hippuric acid, which is probably formed by oxidative decarboxylation of phenylglyoxylic acid, and rabbits have been shown to excrete higher levels of glucuronidated metabolites than humans or rats (ATSDR 1999b).

The elimination of ethylbenzene has been studied in volunteers exposed by inhalation (ATSDR 1999b). The elimination of the major metabolite mandelic acid was reported to be rapid and biphasic, with half-lives of 3.1 hours for the rapid phase and 25 hours for the slow phase (Gromiec and Piotrowski 1984). During an 8-hour inhalation exposure to 4–46 ppm, 23% of the retained ethylbenzene was eliminated in the urine, and 14 hours following termination of exposure, an additional 44% of the retained ethylbenzene was eliminated. The highest excretion rate of urinary metabolites in humans exposed to ethylbenzene by inhalation occurred 6–10 hours after the beginning of exposure and metabolic efficiency was of the exposure dose (Gromiec and Piotrowski 1984; Yamasaki 1984). Data from occupational exposures have generally confirmed these results (Holz et al. 1995; Kawai et al. 1991, 1992; Ogata and Taguchi 1988). In animals, elimination of ethylbenzene metabolites following inhalation or oral exposure is rapid and occurs primarily via urinary metabolites, and to a much lesser degree, via the feces and expired carbon dioxide (ATSDR 1999b). Quantitative and qualitative differences between species were shown to exist in the percentages of metabolites excreted in the urine.

PBPK models have been developed for inhalation exposure to ethylbenzene in rats and humans (Tardif et al. 1997) and for dermal exposure to ethylbenzene in humans (Shatkin and Brown 1991). The dermal model describes the percutaneous absorption of ethylbenzene in dilute aqueous solution.

C.2 Health Effects

Observations in humans and animals indicate that acute high level inhalation exposure to ethylbenzene causes central nervous system effects and irritation of the eyes and respiratory tract that were generally reversible following cessation of exposure (ATSDR 1999b). Information on neurological effects of ethylbenzene in humans is limited to an early report of dizziness accompanied by vertigo following acute exposure to 2,000–5,000 ppm ethylbenzene (Yant et al. 1930). Neurological effects of acute exposure to ≥2,000 ppm in rats, mice, and/or guinea pigs included decreased arousal and rearing, motor disturbances (gait, mobility, and righting reflex), decreased grip strength, increased landing foot splay, impaired psychomotor coordination, and narcotic effects such as prostration and reduced activity (Bio/dynamics 1986; Cragg et al. 1989; Molnar et al. 1986; Tegeris and Balster 1994; Yant et al. 1930). Increased motor activity and sporadic salivation were observed in rats at concentrations of 382–400 ppm and above (Cragg et al. 1989; Molnar et al. 1986). No signs of neurotoxicity were found in rabbits intermittently exposed to 2,400 ppm for 4 days or 1,610 ppm for 4 weeks (Cragg et al. 1989). Changes in dopamine and other biochemical alterations occurred in rats and rabbits exposed to 2,000 and 750 ppm, respectively, for 3–7 days (Andersson et al. 1981; Mutti et al. 1988; Romanelli et al. 1986). Evoked electrical activity in the brain was depressed in rats and mice acutely exposed to 245 and 342 ppm ethylbenzene, respectively (Frantik et al. 1994). Information on oral exposure is limited to a report in which no overt behavioral changes were observed in rats administered ethylbenzene by gavage for 6 months at concentrations ranging from 13.6 to 680 mg/kg/day (Wolf et al. 1956).

Ocular irritation, burning, and lacrimation occurred in humans acutely exposed to ≥1,000 ppm ethylbenzene vapor, and throat irritation and chest congestion were observed at ≥2,000 ppm, and (Cometto-Muniz and Cain 1995; Thienes and Haley 1972; Yant et al. 1930). Animal studies also showed ocular irritation and respiratory effects (e.g., pulmonary congestion and reduced respiratory rate) following acute exposure to concentrations generally above 1,000 ppm (ATSDR 1999b).

Animal data suggest that the liver and kidneys may be a target of toxicity for ethylbenzene. Acute inhalation exposure to high concentrations of ethylbenzene induced effects in the liver that were generally mild and indicative of adaptation (increased metabolism), including increased microsomal enzyme

activity and other biochemical changes, ultrastructural changes, and increased liver weight (Bio/dynamics 1986; Cragg et al. 1989; Elovarra et al. 1985; Wolf et al. 1956). Hepatic histological effects that included necrosis were observed in mice, but not rats, that were exposed to concentrations of ethylbenzene up to 750 ppm for 2 years (NTP 1992). Renal effects manifested as histopathological changes (e.g., tubular swelling and hyperplasia), enzymatic changes, and increased organ weight were observed in a number of species following inhalation exposure to ethylbenzene following acute and longer-term exposure to concentrations generally ≥600 and ≥1,200 ppm, respectively (Andrew et al. 1981; Bio/dynamics 1986; Cragg et al. 1989; NTP 1992, 1996; Wolf et al. 1956).

Inconclusive information is available on the hematotoxic potential of ethylbenzene (ATSDR 1999b). Two studies involving long-term monitoring of workers occupationally exposed to ethylbenzene showed conflicting results with respect to effects on the hematopoietic system. One study (Angerer and Wulf 1985) reported an increase in the number of lymphocytes and a decrease in hemoglobin levels, whereas no adverse hematological effects were reported in the other study (Bardodej and Cirek 1988). There was likely simultaneous exposure to other chemicals in both of these studies. Platelet and leukocyte counts were increased in rats exposed to 782 ppm ethylbenzene for 4 weeks, although no hematological effects were observed in rats or other species exposed to similar or higher concentrations (Cragg et al. 1989; NTP 1992; Wolf et al. 1956).

The reproductive effects of ethylbenzene are incompletely characterized, although there are some data suggesting that the male and female reproductive systems may be a target of toxicity. No multigeneration studies have been performed. No histopathological changes were induced in the testes of rat, mice, or rabbits following inhalation exposure to ≤2,400 ppm ethylbenzene for 4 days, rats and mice exposed to ≤782 ppm for 4 weeks, or rabbits exposed to ≤1,610 ppm for 4 weeks (Bio/dynamics 1986; Cragg et al. 1989). There were no effects on sperm, length of the estrous cycle, or histopathology of reproductive organs in male or female rats or mice exposed to 975 ppm for 90 days, although chronic exposure to 750 ppm caused increased incidences of testicular tumors in rats (NTP 1992, 1999). Ungvary and Tatrai (1985) reported an increase in postimplantation death in the offspring of rats exposed to ≥138 ppm continuously during organogenesis and an increase in abortions in rabbits similarly exposed to 230 ppm. Fertility was reduced in female rats exposed for 3 weeks pre-gestation and during gestation to 1,000 ppm, but the investigators did not consider the effect to be significant (Andrew et al. 1981). Acute oral exposure to 500 or 1,000 mg/kg ethylbenzene decreased peripheral hormone levels and delayed the estrus cycle in female rats during the diestrus stage (Ungvary 1986). Decreased levels of hormones (e.g.,

luteinizing hormone, progesterone, and 17 β-estradiol) were accompanied by uterine changes (increased stromal tissue with dense collagen bundles and reduced lumen), but no dose response was noted.

Developmental toxicity studies in animals indicate that inhalation exposure to ethylbenzene can produce minimal fetotoxic effects at exposure levels that may or may not induce minimal maternal changes (ATSDR 1999b). Effects included skeletal anomalies and supernumerary ribs in offspring of rats that were intermittently exposed to 959 ppm of ethylbenzene during gestation (Andrew et al. 1981). Extra ribs and anomalies of the urinary tract occurred in fetuses of rats continuously exposed to 552 ppm ethylbenzene during gestation, and urinary tract anomalies were also increased in fetal mice similarly exposed to 115 ppm (Ungvary and Tatrai 1985).

Ethylbenzene was not genotoxic in most studies, although some marginal effects have been reported. Workers exposed to low levels of ethylbenzene in a styrene plant showed no increases in sister chromatid exchanges, DNA adduct formation, micronuclei, or DNA single-strand breaks in the peripheral lymphocytes (Holz et al. 1995). Micronucleated peripheral erythrocytes were not increased in mice that were exposed to 750 ppm ethylbenzene for 13 weeks (NTP 1999). Ethylbenzene was generally not mutagenic in bacteria or yeast cells *in vitro*, and did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster cells (ATSDR 1999b). A weak positive response was observed when ethylbenzene was tested for sister chromatid exchanges in human lymphocytes *in vitro*, but only at a concentration that was toxic to the cells (Norppa and Vainio 1983). A positive response also was seen in mouse lymphoma cells when ethylbenzene was tested at a near lethal concentration (McGregor et al. 1988).

The carcinogenicity of ethylbenzene has been examined in three studies: an epidemiological study of humans occupationally exposed by inhalation (Bardodej and Cirek 1988), a chronic inhalation bioassay in rats and mice (Chan et al. 1998; NTP 1999), and a chronic oral study in rats (Maltoni et al. 1985). The results of both the human epidemiological study and the oral study in rats were inconclusive. The inhalation bioassay intermittently exposed rats and mice to $\leq$750 ppm ethylbenzene for 104 weeks and concluded that there was clear evidence of carcinogenic activity in male rats based on increased incidences of renal tubule neoplasms and testicular adenomas (Chan et al. 1998; NTP 1999). The inhalation study also concluded that there was some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenoma, in male mice based on increased incidences of alveolar/bronchiolar neoplasms, and in female mice based on increased incidences of hepatocellular neoplasms.

C.3 Mechanisms of Action

Relatively little information exists regarding the mechanism of ethylbenzene toxicity. Most studies have focused on the possible mechanism of neurotoxicity. *In vitro* studies on the mechanism of toxicity of ethylbenzene have focused on the effect of this chemical on cell membranes, particularly that of the astrocyte (Engelke et al. 1993; Naskali et al. 1993, 1994; Sikkema et al. 1995; Vaalavirta and Tähti 1995a, 1995b). It has been suggested that changes in the structure and integrity of the cell membrane after partitioning of ethylbenzene into the lipid bilayer may be a mechanism of toxicity of ethylbenzene (Sikkema et al. 1995). Changes in the integrity of the cell membrane may subsequently affect the function of membrane, particularly as a barrier and in energy transduction, and in the formation of a matrix for proteins and enzymes. Engelke et al. (1993) showed that incubation of pig liver microsomes with ethylbenzene caused an accumulation of ethylbenzene in the microsomal membrane, which, in turn, increased the fluidity of the membrane. Although incubation of the microsomal membranes with ethylbenzene did not change the content of cytochrome P-450 or cytochrome b_5 content, or the activities of nicotinamide adenine dinucleotide phosphate (oxidized form) (NADPH)-cytochrome P-450 reductase or nicotinamide adenine dinucleotide phosphate (reduced form) (NADH)-cytochrome b_5 reductase, a change in the reduction kinetics of these enzymes was observed. The authors proposed that the observed change in kinetics may be due to a rearrangement of the cytochrome P-450 molecules in the microsomal membrane as a result of the accumulation of ethylbenzene in the membrane.

Vaalavirta and Tähti (1995a, 1995b) and Naskali et al. (1993, 1994) investigated the effect of ethylbenzene on the membrane of the rat astrocyte as an *in vitro* model for the membrane-mediated effects of solvents on the central nervous system. Cultured astrocytes from the cerebellum of neonatal Sprague-Dawley rats were sensitive to the effects of ethylbenzene, as measured by the inhibition of activity of Na^+, K^+-ATPase, and Mg^{++}-ATPase (Vaalavirta and Tähti 1995a, 1995b). This effect was found to be dose-dependent (Naskali et al. 1994). Inhibition of these membrane-bound enzymes that regulate the ion channels of the membrane may disturb the ability of the cells to maintain homeostasis. Experiments with rat synaptosome preparations, similar to those using microsomal preparations by Engelke et al. (1993), showed that membrane fluidity was increased after exposure to ethylbenzene. ATPase and acetylcholinesterase activity were also decreased, as seen in the astrocyte preparations. Results from the *in vitro* studies described above are consistent with what would be expected of the interaction of a lipophilic chemical with cell membranes. However, there is no direct evidence that the interaction of ethylbenzene with astrocytes *in vivo* is the mechanism of neurological effects following acute exposure to high levels of ethylbenzene in humans or in animals.

The mechanism(s) of toxic effects of ethylbenzene on organs or systems other than the nervous system has not been elucidated, but is likely to be related to the formation of reactive metabolites that ultimately bind to cell macromolecules (ATSDR 1999b; NTP 1999).

C.4 Health Guidelines

ATSDR (1999b) derived an intermediate-duration inhalation MRL of 1.0 ppm for ethylbenzene based on a NOAEL of 97 ppm for developmental effects (skeletal anomalies) in rats (Andrew et al. 1981). The animal NOAEL was converted to a $NOAEL_{(HEC)}$ of 97 ppm and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 to account for human variability) to yield the MRL. The animals were exposed for 7 hours/day, 5 days/week for 3 weeks prior to mating and subsequently through gestation day 19.

No acute- or chronic-duration inhalation MRLs were derived for ethylbenzene due to a lack of appropriate data (ATSDR 1999b).

No acute-, intermediate-, or chronic-duration oral MRLs were derived for ethylbenzene due to a lack of appropriate data (ATSDR 1999b).

The EPA IRIS database lists an RfD and an RfC for ethylbenzene (IRIS 2001). The RfD is based on a NOAEL of 136 mg/kg/day (converted to 97 mg/kg/day) for liver and kidney toxicity in rats (Wolf et al. 1956) and an uncertainty factor of 1,000 (10 for subchronic-to-chronic extrapolation, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were exposed to ethylbenzene in olive oil by gavage 5 days/week for 182 days.

The EPA RfC for ethylbenzene is based on a NOAEL of 100 ppm for developmental toxicity in rats and rabbits (Andrew et al. 1981; Hardin et al. 1981) and an uncertainty factor of 300 (3 for extrapolation from animals to humans, 10 for human variability, and 10 for database deficiencies) (IRIS 2001). The animals were exposed 6–7 hours/day during days 1–19 (rats) and days 1–24 (rabbits) of gestation.

ACGIH (2001) recommends a TLV-TWA of 100 ppm and STEL/C of 125 ppm for ethylbenzene based on irritation and central nervous system effects.

The NTP (2001) has not listed ethylbenzene as a known or anticipated human carcinogen. EPA determined that ethylbenzene is not classifiable as to human carcinogenicity (Category D) due to lack of animal bioassay and human data (IRIS 2001). The NTP and EPA assessments predate the positive findings of the NTP (1999) carcinogenesis bioassay in rats and mice. IARC (2000) concluded that ethylbenzene is possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in animals.

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Appendix D: Background Information for Xylenes

This appendix summarizes information on the toxicokinetics, health effects, mechanisms of action, and health guidelines for xylenes. The summaries are mainly based on information presented in the ATSDR (1995) toxicological profile for xylenes and therefore do not represent reviews of the primary literature. The chemical structures for xylenes are included in Appendix E.

D.1 Toxicokinetics

Xylenes are well absorbed by the inhalation and oral routes. Experimental studies with humans found that retention of the various isomers was similar following inhalation of either *o*-, *m*-, or *p*-xylene and averaged 63.6% (Sedivec and Flek 1976). Other investigators have estimated that between 49.8 and 72.8% of inhaled xylene is retained in humans (ATSDR 1995). There appear to be two phases of respiratory absorption; the first is apparently short, occurring within 15 minutes of initiation of exposure, and the second phase is longer (about 1 hour) and represents the establishment of an equilibrium between the inhaled xylene and blood (ATSDR 1995). Oral absorption data are limited, but indicate that almost complete absorption (87–92%) occurred in rabbits following ingestion of a 1.7–1.8 g dose of *o*-, *m*-, or *p*-xylene. Dermal absorption may occur via exposure to xylene vapors as well as through direct dermal contact with the liquid (ATSDR 1995). Xylenes are absorbed dermally to a much lesser extent than by inhalation or oral exposure, especially following dermal exposure to xylene vapor (Riihimaki and Pfaffli 1978). Absorption of *m*-xylene vapor through human skin was determined to be approximately 0.1–2% of that of via inhalation exposure (Riihimaki and Pfaffli 1978). Based on breath sampling and PBPK analysis following vapor exposure, the contribution to *m*-xylene body burden from the dermal route was estimated to be 1.8%. The rate of dermal absorption varied in two human studies of *m*-xylene, ranging from approximately 2 μg/cm^2/minute in one study to 75–160 μg/cm^2/minute in the other (Dutkiewicz and Tyras 1968; Engstrom et al. 1977).

Absorbed xylene is mainly distributed to lipid-rich tissues, particularly adipose and brain (ATSDR 1995). High uptake also occurs in well-perfused organs such as the liver and kidneys. Estimates of the amount of inhaled xylene accumulated in human adipose tissue range from 5 to 10% of the absorbed dose (Astrand 1982; Engstrom and Bjurstrom 1978). The level of xylenes detected in mouse fetal tissues (brain, liver, lung, kidney), which are low in lipids, was only 2% of that found in the maternal brain tissue, which contains large amounts of lipids (Ghantous and Danielsson 1986).

Metabolism of xylenes in humans occurs primarily by hepatic cytochrome P-450-catalyzed oxidation of a side-chain methyl group to yield methylbenzoic acids (*o*-, *m*-, or *p*-toluic acids), which are conjugated with glycine to form methylhippuric acids (ATSDR 1995; Gut et al. 1993; Tassaneeyakul et al. 1996). Important CYP isozymes involved in the methylhydroxylation include CYP2E1 and CYP2B1, and metabolism to methylhippuric acids accounts for almost all (>90%) of the absorbed dose in humans, regardless of the isomer, route of administration, or dose or duration of exposure. Minor metabolic pathways that account for <10% of the absorbed dose in humans include unchanged xylene in the exhaled breath, and methylbenzyl alcohols, *o*-toluic acid glucuronide, xylene mercapturic acid, and xylenols (dimethylphenols) in the urine. CYP1A2 appears to be involved in the formation of the minor phenolic metabolites. The metabolism of xylenes in rats and other laboratory animals is qualitatively similar to that of humans, although glucuronide conjugates make up a larger proportion of the urinary excretion products. A toxic metabolite of xylenes in rats and rabbits that has not been confirmed in humans appears to be methylbenzaldehyde (Carlone and Fouts 1974; Patel et al. 1978; Smith et al. 1982), which is formed by the action of alcohol dehydrogenase on methylbenzyl alcohol in lung and liver tissues. Some studies indicate that the metabolic differences in humans and animals may partly be explained by differences in the size of doses (ATSDR 1995).

In humans, about 95% of absorbed xylene isomers is excreted as urinary metabolites, almost exclusively as methylhippuric acids, with the most of the remaining amount eliminated unchanged in the exhaled air (ATSDR 1995). Less than 0.005 and 2% of the absorbed dose is excreted in the urine unchanged and as xylenols, respectively. There appear to be at least two distinct phases of elimination, a relatively rapid one (1-hour half-life) and a slower one (20-hour half-life, corresponding to elimination from the muscles and adipose tissue). PBPK modeling suggests that the urinary excretion of *m*-methylhippuric acid in humans following inhalation of *m*-xylene is linear at concentrations up to 500 ppm (Kaneko et al. 1991a, 1991b). Humans exposed to 100 or 200 ppm *m*-xylene for 7 hours excreted 54 and 61%, respectively, of the administered dose by 18 hours after exposure ended (Ogata et al. 1970). Following intermittent acute exposure to 23, 69, or 138 ppm *m*-xylene, excretion of *m*-methylhippuric acid peaked 6–8 hours after exposure began and subsequently decreased rapidly so that almost no xylene or methyhippuric acid was detected 24 hours later (Senczuk and Orlowski 1978).

PBPK models have been developed for inhalation exposure to *m*-xylene in rats and humans (Lapare et al. 1993; Tardif et al. 1993b, 1995).

D.2 Health Effects

Health effects of mixed xylenes, *o*-xylene, *m*-xylene, and *p*-xylene appear to be similar, although the individual isomers are not necessarily equal in potency with respect to a particular effect (ATSDR 1995). Studies in humans and animals document that the central nervous system is a major and sensitive target of xylene toxicity by the inhalation and oral routes. Human experimental studies indicate that acute inhalation exposure to 100 ppm mixed xylene or 200 ppm *m*-xylene causes impaired short-term memory, impaired reaction time, performance decrements in numerical ability, and alterations in equilibrium and body balance (Gamberale et al. 1978; Riihimaki and Savolainen 1980; Savolainen and Linnavuo 1979; Savolainen and Riihimaki 1981; Savolainen et al. 1979, 1980, 1984, 1985a, 1985b). Case and occupational studies together provide suggestive supportive evidence that acute and chronic inhalation exposure to xylene or solvent mixtures containing xylene may also be associated with many neurotoxic effects (ATSDR 1995). Neurological effects in orally- or dermally-exposed humans have not been studied. Animal studies provide further evidence that mixed xylenes and individual isomers are neurotoxicants following inhalation exposure at concentrations ranging from 160 to 2,000 ppm. Signs of neurotoxicity observed in rats, mice, and gerbils following acute- and intermediate-duration inhalation exposure to various xylene isomers have included narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, elevated auditory thresholds and hearing loss, and changes in brain enzyme activity and levels of brain proteins (ATSDR 1995). Neurotoxic effects were also induced in the offspring of rats exposed by inhalation during gestation, including impaired performance in tests for neuromotor abilities (Rotarod) and learning and memory (Morris water maze) and delayed ontogeny of the air righting reflex (ATSDR 1995; Hass and Jakobsen 1993). Effects indicative of central nervous system toxicity (e.g., increased latency of visual-evoked potentials, lethargy, tremors, convulsions, respiratory depression, weakness, unsteadiness, and hyperactivity) similarly occurred in animals following acute-, intermediate-, and chronic-duration oral exposure to xylenes, although most of these were serious nonthreshold effects that occurred at high doses (ATSDR 1995).

Xylene is irritating to the to the respiratory tract, eyes, and skin. Adverse respiratory effects that have been observed in humans and animals following acute-, intermediate-, and/or chronic-duration inhalation exposure xylene include nose and throat irritation, labored breathing, and pulmonary congestion, inflammation, and edema (ATSDR 1995). LOAELs for these effects are incompletely characterized, although there are reports of nose and throat irritation following acute-, and chronic-duration inhalation exposures to concentrations of 100–200 and 14 ppm, respectively (Hake et al. 1981; Nelson et al. 1943; Uchida et al. 1993).

Xylene can affect the liver and kidney effects at exposure levels higher than the LOAELs for neurological effects and respiratory irritation. Inhalation or oral exposure to mixed xylenes and/or individual isomers produced hepatic effects in animals that appear to have been generally mild and adaptive in nature, including hepatic cytochrome P-450 content, proliferation of hepatic endoplasmic reticulum, increased liver weight, decreased hepatic glycogen, decreased hexobarbital sleeping time, and/or congestion of liver cells (ATSDR 1995). Occupational studies provide suggestive evidence that humans exposed to high inhalation levels of solvent mixtures containing xylene may be at increased risk of developing renal dysfunction and/or damage (ATSDR 1995). Renal effects such as increased blood urea concentrations, decreased urinary clearance of endogenous creatinine, increased lysozymuria, increased urinary levels of β-glucuronidase, and increased urinary excretion of albumin, erythrocytes, and leukocytes were observed, but the effects are not solely attributable to xylene due to the confounding exposures. Kidney effects found in inhalation and oral studies in animals included increased renal enzyme activity, increased cytochrome P-450 content, and increased kidney weight without histopathological alterations.

Human and animal data provide no indications of adverse hematological effects following inhalation of xylene. In the past, chronic occupational exposure to xylene was thought to be associated with a variety of hematological effects. However, exposure in all cases was to solvent mixtures known or suspected to contain benzene, and because benzene causes leukemia and other blood dyscrasias in humans, these effects cannot be attributed solely to xylene (ATSDR 1995). Hematological effects were not observed in an occupational study of xylene-exposed workers in which no benzene was involved (Uchida et al. 1993), or in rats, dogs, or guinea pigs exposed by inhalation to 810 ppm mixed xylenes or 780 ppm *o*-xylene for intermediate durations (Carpenter et al. 1975; Jenkins et al. 1970).

Information on the developmental toxicity of xylenes in humans is limited to a few occupational studies that are inadequate for assessing the relationship between exposure to xylenes and developmental effects due to concurrent exposure to other solvents and small numbers of subjects (ATSDR 1995). In animals, inhalation exposure to mixed xylenes (500 ppm) or the individual isomers (350–700 ppm) produced fetotoxic effects in rats, mice, and rabbits, including increased incidences of skeletal variations in fetuses, delayed ossification, fetal resorptions, hemorrhages in fetal organs, and decreased fetal body weight (Balogh et al. 1982; Bio/dynamics 1983; Hass and Jakobsen 1993; Hudak and Ungvary 1978; Litton Bionetics 1978; Mirkova et al. 1983; Ungvary 1985; Ungvary and Tatrai 1985; Ungvary et al. 1980, 1981). The levels at which these effects occurred were generally maternotoxic (ATSDR 1995). Milder neurodevelopmental effects occurred in rats at lower gestational inhalation exposure levels (200–500 ppm) that did not induce maternal toxicity, including impaired performance in tests for

neuromotor (Rotarod) and learning and memory (Morris water maze) abilities, and delayed ontogeny of the air righting reflex (ATSDR 1995; Hass and Jakobsen 1993). A LOAEL of 200 ppm for impaired Rotarod performance was identified in 1- and 2-day-old rat pups that were intermittently exposed to mixed xylenes during gestation (Hass and Jakobsen 1993). An oral study found that mixed xylenes-induced cleft plate and decreased fetal weight in mice exposed to maternally toxic doses (Marks et al. 1982), and a dermal study found changes in brain enzymes (cholinesterase, cytochrome) in the brain of fetal rats (Mirkova et al. 1979).

Information on the reproductive toxicity of xylenes in humans is limited to two studies that found an increased incidence of spontaneous abortions following paternal exposure (Taskinen et al. 1989) or maternal exposure (Taskinen et al. 1994). These findings are inconclusive due to study limitations including multiple chemical exposures and small population size. No reproductive effects were found in rats following inhalation of 500 ppm mixed xylenes from before mating through lactation (Bio/dynamics 1983), but only one generation was assessed and this was primarily a developmental toxicity study. No changes in the histology of the testes and accessory glands or circulating male hormone levels occurred in rats following exposure to 1,000 ppm mixed xylenes for 61 days (Nylen et al. 1989). No histopathological changes were found in reproductive tissues of male and female rats and mice following intermediate- or chronic-duration oral exposure to mixed xylene or its isomers at doses as high as 500–2,000 mg/kg/day (NTP 1986; Wolfe 1988a, 1988b).

Human carcinogenicity data consist of limited and inconclusive occupational studies that examined risks of cancer and leukemia among workers exposed to xylenes together with other solvents (Arp et al. 1983; Wilkosky et al. 1984). Animal carcinogenicity data for xylenes are limited to equivocal oral studies with mixed xylenes (Maltoni et al. 1983, 1985; NTP 1986), and dermal initiation/promotion studies suggesting that xylene may be a promoter for skin cancer and might also act as an initiator or cocarcinogen (Berenblum 1941; Pound 1970; Pound and Withers 1963). No animal carcinogenicity data for xylenes are available for inhalation exposure. The insufficiencies of the available data preclude concluding that there is a causal relationship between xylene exposure and cancer. Mixed xylenes and the individual xylene isomers have been tested for genotoxicity in a variety of *in vitro* and *in vivo* assays with predominantly negative results, indicating that xylenes are nongenotoxic (ATSDR 1995).

D.3 Mechanisms of Action

The mechanisms by which xylene exerts its toxic effects are not completely understood, although a number of theories exist pertaining to induction of effects in the nervous system, developing fetus, lung, and kidney. The central nervous system toxicity observed during exposure to high concentrations of xylene has been attributed to the liposolubility of xylene in the neuronal membrane (Desi et al. 1967; EPA 1985; Gerarde 1959; Savolainen and Pfaffli 1980; Tahti 1992). It has been suggested that xylene disturbs the action of proteins essential to normal neuronal function. This is similar to the way general anesthetic agents work, (i.e., either by a disruption of the lipid environment in which membrane proteins function or by direct interaction with the hydrophobic/hydrophilic conformation of proteins in the membranes). Changes in levels of various neurotransmitters and lipid composition have been observed in several brain areas following acute- and intermediate-duration exposure to xylene (Andersson et al. 1981; Honma et al. 1983; Savolainen and Seppalainen 1979). It is unclear whether these represent direct effects of xylene or are secondary changes resulting from nonspecific central nervous system depression. Some authors have also suggested that metabolic intermediates, such as arene oxides or methylbenzaldehyde, may be responsible for the toxic effects of xylene (Savolainen and Pfaffli 1980). Oxidation of xylene to these intermediates by microsomal enzyme systems may occur within brain cells (Savolainen and Pfaffli 1980).

Inhibition of pulmonary microsomal enzymes has been observed by several investigators (Elovaara et al. 1987; Patel et al. 1978; Silverman and Schatz 1991; Smith et al. 1982; Stickney et al. 1989). The exact mechanism of the enzyme inhibition is unknown, but has been attributed to the formation of a toxic reactive metabolite (such as methylbenzaldehyde) that binds directly to microsomal protein and inactivates the microsomal enzymes (Patel et al. 1978; Smith et al. 1982). Direct effects on microsomal membrane fluidity and/or lipid content do not appear to be involved (Stickney et al. 1989).

The mechanism for xylene-related renal toxicity is unknown, but may be related to the formation of reactive metabolites and subsequent irritation or direct membrane fluidization (EPA 1985). In humans exposed to solvent mixtures containing xylene, the increased urinary levels of β-glucuronidase have been proposed to be due to a faster cellular turnover in the renal tubular epithelium because of a mild toxicity (Franchini et al. 1983). The lysozymuria and increase in urinary excretion of albumin may be indicative of potential damage to the renal tubules and renal glomeruli, respectively (Askergren 1982; Franchini et al. 1983).

The exact mechanism by which mixed xylenes produce toxic effects in fetuses has not been fully investigated. The information on mechanisms of neurotoxicity in the preceding paragraph is relevant to exposed offspring as well as adults. Based on results of studies with rats, *p*-xylene-induced delayed fetal development may be related to decreased levels of progesterone and estradiol (Ungvary et al. 1981). The reduced levels of these hormones may have been due to increased microsomal enzyme activity and increased hormone catabolism.

D.4 Health Guidelines

ATSDR (1995) derived an acute-duration inhalation MRL of 1 ppm for mixed xylenes based on a LOAEL of 100 ppm for neurological effects (increased reaction times) in humans (Dudek et al. 1990) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability). Volunteers were exposed to xylenes for 4 hours.

ATSDR (1995) derived an intermediate-duration inhalation MRL of 0.7 ppm for mixed xylenes based on a LOAEL of 200 ppm for neurodevelopmental effects (reduced rotarod performance in offspring) in rats (Hass and Jakobsen 1993) and an uncertainty factor of 300 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). The animals were exposed 6 hours/day on gestation days 4–20.

ATSDR (1995) derived a chronic-duration inhalation MRL of 0.1 ppm for mixed xylenes based on a LOAEL of 14 ppm for subjective neurological effects (symptoms including anxiety, forgetfulness, and inability to concentrate) and eye and respiratory tract irritation in humans (Uchida et al. 1993) and an uncertainty factor of 100 (10 for use of a LOAEL and 10 human variability). Workers were occupationally exposed to xylenes for an average of 7 years.

ATSDR (1995) derived an acute-duration oral MRL of 1 mg/kg/day for *p*-xylene based on a NOAEL of 125 mg/kg/day for neurological effects (altered visual evoked potentials) in rats (Dyer et al.1988) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The animals were administered a single dose of *p*-xylene in oil.

ATSDR (1995) derived an intermediate-duration oral MRL of 0.2 mg/kg/day for mixed xylenes based on a LOAEL of 150 mg/kg/day for renal toxicity (early chronic nephropathy) in rats (Condie et al. 1988) and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and

10 for human variability). The animals were administered mixed xylenes in oil by gavage daily for 90 days.

ATSDR (1995) derived an intermediate-duration oral MRL of 0.6 mg/kg/day for *m*-xylene based on a LOAEL of 800 mg/kg/day for hepatic toxicity (increased plasma alanine aminotransferase and plasma membrane damage) in rats (Elovaara et al. 1989) and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). The animals were administered *m*-xylene in oil by gavage 5 days/week for 3.3 weeks.

ATSDR (1995) did not derive oral MRLs for *o*-xylene for any duration period, or chronic-duration oral MRLs for mixed xylenes or any xylene isomer, due to lack of suitable data.

The EPA IRIS database lists an oral RfD, but no inhalation RfC for xylenes (IRIS 2001). The RfD is based on a NOAEL of 250 mg/kg/day (converted to 179 mg/kg/day) for neurological effects in rats (NTP 1986) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). The animals were administered mixed xylenes by gavage 5 days/week for 103 weeks. ACGIH (2001) recommends a TLV-TWA of 100 ppm and STEL/C of 150 ppm for xylene (*o*-, *m*-, and *p*-isomers) based on irritation in humans.

The NTP (2001) has not listed xylenes as a known or anticipated human carcinogen. EPA determined that mixed xylenes are not classifiable as to human carcinogenicity (Category D) based on no human data and inadequate animal data (IRIS 2001). IARC (1999b) concluded that xylenes are not classifiable as to their carcinogenicity (Group 3) based on inadequate evidence in humans and animals.

D.5 References

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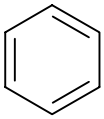
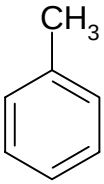
Appendix E: Chemical Structures of Mixture Components

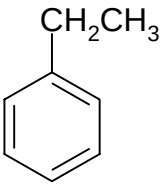
Benzene



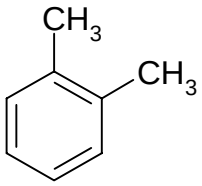
Toluene



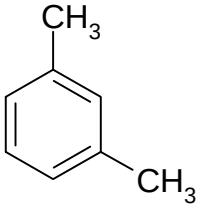
Ethylbenzene



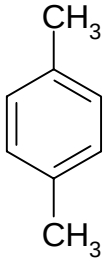
Xylenes



o-xylene



m-xylene



p-xylene