2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO XYLENE IN THE UNITED STATES

Xylenes (mixtures of *ortho*-, *meta*-, and *para*-isomers) are used as industrial solvents, synthetic intermediates, and solvents in commercial products such as paints, coatings, adhesive removers, and paint thinners; they are also a component of gasoline. Xylenes are released to the atmosphere primarily as fugitive emissions from industrial sources (e.g., petroleum refineries, chemical plants), in automobile exhaust, and through volatilization from their use as solvents. Discharges into waterways and spills on land result primarily from use, storage, and transport of petroleum products and waste disposal.

When xylene is released to soil or surface water, it is expected to volatilize into the atmosphere where it is quickly degraded. The half-life for xylene in the atmosphere is 8–14 hours. Any xylene in soil or surface water that does not volatilize quickly may undergo biodegradation. Xylene may also leach into groundwater where biodegradation becomes the primary removal process. Half-lives measured for xylene in groundwater range from 25 to 287 days. Degradation rates in groundwater will vary depending on differences in conditions such as temperature, presence of oxygen, and presence of electron acceptors.

Xylene is primarily detected in air. Typical xylene concentrations range from 1 to 30 ppb in outdoor air and from 1 to 10 ppb in indoor air. Xylene has been detected in <5% of samples collected during groundwater surveys conducted in the United States. Median xylene concentrations of ≤2 ppb have been reported in 406 urban and 2,542 rural drinking water wells or monitoring wells in the United States. Less than 6% of drinking water samples collected during drinking water surveys contained xylene; the mean concentrations in positive samples were typically <2 ppb. Xylene has been detected in all kinds of foods (e.g., meat, fruit, dairy, fish, vegetables, grains), with typical concentrations ranging from 1 to 100 ppb.

Because individual xylene isomers are used in large amounts in industrial settings, people who work at or live near these locations may receive a higher exposure to one xylene isomer compared to the other isomers. However, since xylenes are present as a mixture in gasoline and in the solvent components of commonly used commercial products (paint, coatings, etc.), exposure of the general population is expected to be primarily to xylenes as a mixture, and not to the separate xylene isomers.

Inhalation appears to be the major route of exposure to xylene, although exposure through the use or consumption of groundwater is also likely in areas where there is subsurface gasoline contamination.

During exposures to xylene vapor, a small amount of dermal absorption also occurs, ≤2% of the amount inhaled. Humans may also be exposed to xylene through smoking, consumption of xylene-contaminated foods, and dermal contact with consumer products containing xylene. Children are expected to be exposed to xylene by the same routes as adults. Since xylene has a low affinity for adsorption onto soil and dust particles and a high volatilization rate, the risk of exposure for small children from ingesting soil or dust is likely to be low. The average daily intake of total xylene (sum of *o*-, *m*-, and *p*-xylene intakes) for the general population is estimated as 0.3–8.6 µg/kg/day from inhalation exposure and 0.06 µg/kg/day from ingestion of drinking water assuming typical low background levels. Based on a maximal concentration of 1.5 mg/L in drinking water, the maximal daily consumption of xylenes from drinking water would be 0.04 mg/kg/day. These exposure levels are below the minimal risk levels (MRLs) established for xylenes (see Section 2.3 and Appendix A).

See Chapter 6 for more detailed information regarding concentrations of xylenes in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

Xylenes, because of their lipophilic properties, are rapidly absorbed by all routes of exposure, rapidly distributed throughout the body, and, if not metabolized, quickly eliminated in exhaled air. In humans, absorption has been estimated as >50% through the lungs following inhalation exposure and <50% through the gastrointestinal system. In humans exposed by inhalation, up to 2% of the absorbed dose may be absorbed through the skin. The major pathway for metabolism involves mixed function oxidases in the liver, resulting mainly in the formation of isomers of methylhippuric acid that are eliminated in the urine and are used as an index of exposure for occupational monitoring. Background urinary levels of methylhippuric acids in nonexposed workers are <2 mg/g creatinine. Xylenes tend not to accumulate in the body, but they may be sequestered briefly in fat tissues due to their lipophilicity; elimination of xylene is slower in individuals with a greater percentage of body fat. The primary effects of xylene exposure involve the nervous system by all routes of exposure, the respiratory tract by inhalation exposure, and, at higher oral exposure levels, hepatic, renal, and body weight effects. No adverse health effects have been associated with the background levels of xylene to which the general population is typically exposed (see Section 2.1). Isomers of xylene have similar toxicokinetic properties and elicit similar toxicological effects, with no single isomer consistently exhibiting the greatest potency, depending on the end point. This issue is discussed further in the introduction to Section 2.3 and in Section 3.2.

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Information on the toxicity of xylene in humans comes from case reports, occupational studies, and studies on volunteers. At acute-duration inhalation concentrations as low as 50 ppm, xylenes produce irritant effects on the eyes, skin, and mucous membranes; impaired respiratory function; and mild central nervous system effects, including headache and dizziness. Increases in subjective reports of eye irritation, sore throat, and neurological effects (anxiety, forgetfulness, inability to concentrate, and a sensation of intoxication) were noted following chronic-duration occupational exposure at 14 ppm. Irritation of the eye may occur from contact with xylene vapor or from direct contact with xylene liquid, in which case photophobia, redness of the conjunctiva, and partial loss of the conjunctival and corneal epithelia have been reported. Slight-to-moderate eye irritation has also been observed in rabbits following direct instillation with ≥23 mg/kg mixed xylenes. With increasing airborne xylene concentrations of 100–400 ppm, other neurological effects reported in acutely exposed human subjects include retardation of response times and impairments in memory and body balance. Acute exposure to an estimated 10,000 ppm xylenes elicited tremors, mental confusion, and depressant effects (narcosis) on the central nervous system that caused at least one fatality due to respiratory failure. All of these effects are related to the lipophilic properties of xylenes, which interfere with the integrity of cell membranes and alter neuronal function. In addition to neurological and respiratory effects, an increase in the reporting of nausea was noted following controlled exposure to *m*-xylene at 50 ppm. Symptoms of nausea and vomiting have also been noted in workers exposed to xylene vapors.

Other effects of xylene exposure involve the liver and kidney in humans and animals and body weight effects in laboratory animals. Hepatic effects (elevated serum transaminases and hepatocellular vacuolation) were observed in a limited number of case reports describing effects of acute exposure to an estimated 700–10,000 ppm mixed xylene, but were not observed in workers with chronic occupational exposure at 14 ppm. Hepatic effects in laboratory animals exposed orally at ≥750 mg/kg/day or by inhalation at ≥300 ppm include increases in liver weight, serum enzyme levels, and cytochrome P-450 levels, but no histopathology. However, a number of authors characterized the hepatic effects in animals as adaptive rather than adverse. Information on renal effects (distal renal tubular acidemia and abnormal clinical chemistry values) of xylene in acutely exposed humans is confounded by exposure to other compounds or uncertainties as to the duration of exposure. No alterations in renal serum biochemistry values were observed in workers exposed to 14 ppm mixed xylene for several years. Renal effects in repeatedly exposed laboratory animals include increases in renal enzyme activity, cytochrome P-450 content, and increased kidney-to-body-weight ratios following inhalation exposure at 50–2,000 ppm or oral exposure and increased chronic nephropathy in rats exposed at ≥750 mg/kg/day. However, no renal effects were observed in rats exposed via inhalation to 810 ppm mixed xylenes for 13 weeks, gavage

doses of 1,000 mg/kg/day 5 days/week for 13 weeks, or gavage doses of 800 mg/kg/day to *m*- or *p*-xylene for 90 consecutive days. It is not known whether the variability in induction of renal effects from mixed xylene is related to variations in the relative amounts of *o*-xylene or ethylbenzene or variations in the strains of rats tested. Decreased body weight gain has been observed in laboratory animals repeatedly exposed by inhalation at ≥700 ppm or by oral dosing or at ≥700 mg/kg/day; a 5–8% reduction in body weight gain observed in male rats exposed at 500 mg/kg during the last year of a 2-year study is not considered biologically significant. Dermal exposure of humans to xylene causes skin irritation, dryness and scaling of the skin, and vasodilation. In addition, one case report demonstrated the possibility that contact urticaria can develop after several months of occupational exposure to 100 ppm xylene vapors. Dermal effects of *m*-xylene, *o*-xylene, or mixed xylenes in laboratory animal studies included skin irritation (erythema and edema) at topical doses as low as 2.3 mg/kg and more serious effects (eschar formation in some animals and epidermal thickening) at topical doses of ≥114 mg/kg. Rat skin that developed moderate erythema after treatment with *m*- or *o*-xylene exhibited increases in transepidermal water loss and increases in pro-inflammatory cytokines (interleukin 1-alpha and tumor necrosis factor-alpha).

Available studies of developmental or reproductive toxicity from occupational exposure to xylenes are not definitive because of the small number of subjects and/or concurrent exposure to other chemicals. In general, developmental studies in animals reported adverse fetal effects only at concentrations that caused maternal toxicity. Developmental effects in laboratory animals exposed to ≥350 ppm xylenes by inhalation include delayed ossification of the skeleton at maternally toxic concentrations and reduced fetal body weight, which is also influenced by maternal body weight effects. Postnatal neurobehavioral deficits (decreased rotarod performance) have been observed in rats gestationally exposed to *m*-xylene at 500 ppm. Oral exposure to 2,060 mg/kg/day of mixed xylene has been associated with cleft plate and decreased fetal weight. Dermal exposure of rats to xylene has been associated with biochemical changes in fetal and maternal brain tissue. No reproductive effects were found in rats following inhalation of 500 ppm xylene before mating and during gestation and lactation. Histopathological examination following intermediate and chronic oral bioassays revealed no adverse effects on the reproductive organs of rats and mice dosed with mixed xylene 5 days/week at 800 and 1,000 mg/kg/day, respectively.

There is no definitive evidence for carcinogenic effects of xylene in humans. Epidemiological studies looking for associations with xylene exposure and specific cancers either reported no cases or a limited number of cases exposed to xylene and/or reported concurrent exposure to multiple solvents. Two-year cancer bioassays in rats and mice exposed by oral gavage provided no evidence for carcinogenicity of

mixed xylene. The Department of Health and Human Services (DHHS) has not classified xylene as to its carcinogenicity. Both IARC and EPA have determined that xylene is not classifiable as to its carcinogenicity in humans, due to inadequate evidence for the carcinogenicity of xylenes in humans and animals.

The following sections discuss the most sensitive effects of exposure to xylenes: neurological and respiratory. These effects, as well as other adverse effects, are discussed in greater detail in Chapter 3.

Neurological Effects. The neurotoxicity of xylenes has been examined in short- and long-term inhalation studies in humans and animals and acute-duration oral studies in animals and appears to be related to the interference of unmetabolized xylene with neuronal membranes. Mild central nervous system effects (subjective symptoms of intoxication, headache, fatigue, and dizziness) have been observed following acute-duration exposure of humans to *m*-xylene at 50 ppm and chronic-duration occupational exposure to mixed xylene at 14 ppm. Results of experimental studies with humans 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. These experimental studies are supported by case reports and occupational studies that described similar neurological effects at higher exposure levels. Isolated cases of unconsciousness, amnesia, brain hemorrhage, and seizures have been associated with accidental acute inhalation exposure to unknown concentrations of xylene (estimated in one case as 10,000 ppm).

Neurological impairment has also been reported in experimental studies with laboratory animals with a similar range of concentration-related severities as in humans. The range of thresholds for specific neurological end points suggests that specific neuronal pathways or regions of the central nervous system may vary in their vulnerability to xylene exposure. Studies in animals have shown that mixed xylene and individual isomers are neurotoxic at airborne concentrations ranging from 50 to 2,000 ppm. A decreased latency of the paw-lick response was reported in rats exposed to 50 ppm *m*-xylene for 3 months. Decreased motor performance and impaired learning have been reported in rats exposed to concentrations between 100 and 3,000 ppm. Loss of cochlear hair cells and/or hearing deficits have been observed in rats exposed by inhalation acutely to ≥1,450 ppm mixed xylene and in rats exposed for 13 weeks to 900 ppm *p*-xylene, but not other isomers or 1,000 ppm mixed xylenes. Changes in the levels of brain enzyme activities or neurotransmitters have been noted with acute exposure to 2,000 ppm with *m*- or *o*-xylenes. Severe neurotoxicity was observed in rats, mice, and gerbils following acute- or intermediate-duration inhalation exposure to the various xylene isomers at concentrations in excess of 1,000 ppm.

These effects included narcosis, prostration, incoordination, tremors, muscular spasms, and labored respiration. All three xylene isomers elicited biphasic response rates in operant behavior studies in mice exposed for 30 minutes: increased responses at ≥1,400 ppm and half-maximal responses at 5,179–6,176 ppm. Motor coordination was impaired at concentrations above 2,000 ppm for the *para* isomer and above 3,000 ppm for the *meta* and *ortho* isomers.

Animal studies have shown that oral exposure to xylenes at high concentrations (single doses of ≥4,000 mg/kg or repeated dosing at 2,000 mg/kg/day for 2 weeks) may result in nervous system effects such as tremors, respiratory depression, weakness, lethargy, unsteadiness, and hyperactivity. Hyperactivity was also observed in all mice after oral dosing with 1,000 mg/kg mixed xylene during weeks 4–103. Rats dosed with *p*-xylene, but not *m*- or *o*-xylene at 900 mg/kg/day, 5 days/week for 2 weeks, experienced significant loss of cochlear hair cells associated with hearing at medium frequencies (10–25 kHz).

Respiratory Effects. Numerous studies in humans identified the respiratory tract as a sensitive target of xylenes following short- or long-term inhalation exposure. Subjective symptoms of nose and throat irritation were noted following single exposures to xylene vapor at concentrations between 50 and 700 ppm, repeated intermediate-duration exposure at 100 ppm, or chronic-duration occupational exposure at 14 ppm. Mild increases in the subjective severity scores for breathing difficulty and small changes in measured pulmonary physiology parameters (reduced forced vital capacity) were observed following acute-duration exposure to *m*-xylene at 50 ppm. Labored breathing with impaired pulmonary function was reported following chronic occupational exposure to unspecified concentrations of xylene vapor. Severe lung congestion with pulmonary hemorrhages and edema was noted in a worker who died following inhalation of paint fumes containing an estimated 10,000 ppm xylene for an undetermined time (<18 hours) and in one case of suicide by ingestion of an undetermined amount of xylene.

Animal data from acute- and intermediate-duration inhalation studies provide supporting evidence for the respiratory effects observed in humans following inhalation exposure to xylene. Respiratory effects noted in laboratory animals exposed for a few minutes at concentrations of ≥690 ppm include a 50% reduction in respiratory rate, labored breathing, irritation of the upper respiratory tract, pulmonary edema, and pulmonary inflammation. Irritation of the upper respiratory tract induced a reflex pause in the expiratory phase of respiration that resulted in an overall reduction in respiratory rate. Decreased metabolic capacity of the lungs was reported to be related to 45–80% decreases in the activities of metabolizing enzymes (cytochrome P-450) following acute exposure to mixed xylenes or individual isomers at concentrations

between 75 and 2,000 ppm. The toxicological significance of the reduction in metabolizing enzymes is not established, but may be related to the lessened ability to reduce cellular concentrations of xylene.

2.3 MINIMAL RISK LEVELS (MRLs)

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

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

Toxicological data from comparative studies demonstrate that, in some cases, the effects and effect levels of the three isomers are similar; e.g., body weight findings in the acute oral study by Condie et al. (1988) or the alveolar concentration levels associated with anaesthetic effects as described by Fang et al. (1996). Other studies have indicated different orders of relative toxicity for the isomers, but there is no consistent pattern indicating that a particular isomer is the most potent for all end points, and the differences in effect levels among the isomers may be small. For example, the *ortho* isomer was most potent in assays on operant behavior (Moser et al. 1985) and motor coordination in rats (Korsak et al. 1990) and in a developmental toxicity assay in rats in which mixed xylenes had the same effect levels (Saillenfait et al. 2003). The *para* isomer, however, was most potent in a different test for motor performance, the inverted screen test (Moser et al. 1985), and in ototoxicity assays in rats (Gagnaire et al. 2001). Given the lack of consistency among the different end points, the most sensitive effect by mixed xylenes or any isomer was chosen as the basis for MRL for mixed xylenes and all isomers for that duration and route of exposure.

Inhalation MRLs

- An MRL of 2 ppm has been derived for acute-duration inhalation exposure (14 days or less) to mixed xylenes

The database for acute-duration inhalation exposure to xylenes includes a number of studies in humans and laboratory animals. Studies in humans identify neurological and respiratory effects as the most sensitive end points for acute-duration inhalation exposure. Effects observed in humans at 50–690 ppm included irritation of the respiratory tract (breathing difficulty, discomfort in nose and throat, reduced forced air capacity), neurotoxicity (dizziness, headache, impaired short-term memory, increase in reaction times), and eye irritation (Carpenter et al. 1975a; Dudek et al. 1990; Gamberale et al. 1978; Nelson et al. 1943; NIOSH 1981). Other neurological effects in humans included altered visual evoked potentials following repeated exposure to 200 ppm *m*-xylene and impaired body balance following a single 4-hour exposure at 400 ppm (Savolainen et al. 1984; Seppalainen et al. 1989).

Acute-duration neurological effects in animals were observed at concentrations of 113 ppm and higher. These included transiently decreased operant responses in rats repeatedly exposed to 113 ppm mixed xylene (Ghosh et al. 1987), altered responses to electric shock in rats and mice exposed once to 230–320 ppm *o*-xylene (Vodickova et al. 1995), and decreased axonal transport in rats repeatedly exposed to 800 ppm mixed xylene (Padilla and Lyerly 1989). Effects related to motor incoordination were observed following exposures to mixed xylenes or individual isomers in the range of 1,010–1,982 ppm (Carpenter et al. 1975a; De Ceaurriz et al. 1983; Korsak et al. 1988, 1990, 1993). Sensory-related changes (altered visual- or auditory-evoked potentials or hearing losses) occurred at exposures in the range of 1,400–1,600 ppm *p*-xylene (Crofton et al. 1994; Dyer et al. 1988; Pryor et al. 1987; Rebert et al. 1995). Other neurological effects included disturbances in brain catecholamine or dopamine following repeated exposure to mixed xylene or individual isomers at 2,000 ppm (Andersson et al. 1981), narcosis in rats at ≥1,940 ppm (Molnar et al. 1986), and ataxia and seizures in cats exposed to 9,500 ppm mixed xylene (Carpenter et al. 1975a).

The lowest effect levels for other end points affected by acute-duration inhalation exposure in animals were higher than the lowest-observed-adverse-effect level (LOAEL) for neurological effects. Rat developmental effects (reduced fetal body weight and delayed ossification) were observed at xylene exposures in the range of 350–2,000 ppm (Saillenfait et al. 2003; Ungvary et al. 1980b); maternal body weight effects were observed at 700 ppm (Ungvary et al. 1980b). Acute-duration respiratory effects in

animals include decreases in lung surfactant following exposure to mixed xylenes or individual isomers at 1,000 ppm or higher (Elovaara et al. 1987; Patel et al. 1978; Toftgard and Nilsen 1982) and decreased respiratory rates in mice briefly exposed to *m*- or *o*-xylene at concentrations of 1,361–2,700 ppm (De Ceaurriz et al. 1981; Korsak et al. 1990, 1991, 1993). Increased mortality has been reported in rodents repeatedly exposed to at 700 ppm (Ungvary et al. 1980b) or once to ≥2,010 ppm (Bonnet et al. 1979; Cameron et al. 1938; Carpenter et al. 1975a; Harper et al. 1975; Hine and Zuidema 1970).

Neurological and respiratory effects are selected as co-critical effects of acute-duration inhalation exposure since they occurred at the lowest tested exposure level of 50 ppm. Ernstgard et al. (2002) was selected as the principal study for acute-duration inhalation exposure because it provides the lowest LOAEL for the co-critical effects. In this study, 28 men and 28 women were exposed to 0 or 50 ppm *m*-xylene in sessions separated by 2 weeks. There were difficulties with recruitment for the study; only 10%/sex out of the original selected group of 1,000/sex agreed to participate and the drop-out rate was high (~72%) because of failure to pass the screening medical examination or a change in life circumstances. Strengths of the study include that subjects served as their own controls, exposures were controlled (with respect to solvent concentration, duration, temperature, and humidity), results were analyzed for statistical significance, and a third exposure condition (150 ppm 2-propanol) resulted in a qualitatively different pattern of adverse effects compared to *m*-xylene exposure. During exposure (3, 60, and 118 minutes from the start of exposure) and post-exposure (140 and 350 minutes from onset), the subjects rated the level of perceived discomfort using a visual analogue scale (0–100 mm) in a questionnaire with 10 questions. Pulmonary function measurements were conducted prior to exposure, immediately after exposure, and 3 hours post-exposure. Nasal swelling was assessed by acoustic rhinometry before, immediately after, and 3 hours after the end of exposure. Nasal lavages obtained before and 3 hours after the end of exposure were evaluated for markers of inflammation. Eye blinking was measured throughout exposure by electromyography, and color vision was assessed before, immediately after, and 3 hours post-exposure. Compared to air-exposure, exposure to *m*-xylene at 50 ppm resulted in small statistically significant increases in the average rating of self-reported symptoms for local irritant effects (discomfort in the eyes and nose), other respiratory effects (breathing difficulty in both sexes and discomfort in the throat or airways in women), and neurological effects (feeling of intoxication in both sexes, and headache, fatigue, and dizziness in men). All self-reported symptoms were characterized as minimal. Although the subjects were not blinded with respect to the exposure conditions, there was only a weak correlation between ratings of smell and ratings of symptoms; the authors conclude that it was unlikely that the perceived exposure by itself significantly affected symptom ratings. Small statistically significant changes were observed in objective measures of respiratory

function in exposed women, but not men, 3 hours after the end of the exposure to *m*-xylene; forced vital capacity was reduced by 2.81% (45-fold more than air exposure) and other parameters dependent on forced vital capacity were likewise altered. The authors indicated that these measured respiratory effects in women were of uncertain significance, but could be explained by possible effects of estrogen or the narrower airways in women compared to men. A minimal LOAEL of 50 ppm is identified for neurological and respiratory effects in humans exposed to *m*-xylene. An uncertainty factor of 30 was applied to the LOAEL of 50 ppm (3 for the use of a minimal LOAEL and 10 for human variability). The resulting MRL of 2 ppm is considered to be protective to human health under acute-duration (≤14 days) inhalation exposures to mixed xylenes or individual isomers.

- An MRL of 0.6 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to mixed xylenes.

The database for intermediate-duration inhalation to xylenes includes one controlled-exposure study in humans and several animal bioassays. Effects in humans exposed to 100–150 ppm *p*-xylene for 1–7.5 hours/day, 5 days/week for 4 weeks included increased reporting of subjective symptoms of irritation of the nose and throat (NIOSH 1981). This study found no significant alterations in objective measures of neurological function (electroencephalography, tests of motor activity, and cognitive performance) and no alterations in pulmonary function in human subjects, but the study was limited in that some group sizes were small (n=2). Animal toxicity bioassays that tested at concentrations below 100 ppm reported no clinical signs of neurotoxicity or no adverse effects liver enzymes or brain weight (Hillefors-Berglund et al. 1995; Jajte et al. 2003; Jenkins et al. 1970). In a special neurobehavioral assay, a LOAEL of 50 ppm was identified for reduced mean latency of the paw-lick response (indicative of increased sensitivity to pain) in rats exposed to *m*-xylene for 3 months (Korsak et al. 1994). Other neurobehavioral effects (impaired rotarod performance, passive avoidance learning) were observed following exposure to 100 ppm *m*-xylene for 4 weeks to 6 months (Gralewicz and Wiaderna 2001; Gralewicz et al. 1995; Korsak et al. 1994); some neurological impairment persisted for 5–9 weeks after exposure (Gralewicz and Wiaderna 2001). Other neurological effects included distribution of astroglia cells in the brain of Mongolian gerbils exposed to 160 ppm mixed xylene for 4 months (Rosengren et al. 1986), delayed maxillary ossification and impaired rotarod performance in rats gestationally exposed to 200 ppm technical-grade xylene for 6 hours/day (Hass and Jakobsen 1993), and impaired motor coordination and spatial orientation following gestational exposure at 500 ppm (Hass et al. 1995, 1997). Neurological effects at higher concentrations included tremors in dogs exposed to 780 ppm *o*-xylene for 6 hours/day, 5 days/week for 6 weeks (Jenkins et al. 1970), alterations in brain neurotransmitters following continuous exposure to 800 ppm mixed xylenes for

30 days (Honma et al. 1983), and auditory effects in rats (hearing loss or decreases in auditory brainstem responses) following exposure to ≥800 ppm mixed xylenes or 900 ppm *p*-xylene for 13 weeks (Gagnaire et al. 2001, 2006; Nylen and Hagman 1994; Pryor et al. 1987).

Non-neurological effects were observed at higher concentrations. Cardiovascular effects (increased thickness of coronary microvessels) were observed in rats exposed to 230 ppm mixed xylenes 6 hours/day, 5 days/week for 4 weeks (Morvai et al. 1987). Hepatic effects (increased liver weight) were observed at a LOAEL of 600 ppm in rats discontinuously exposed to mixed xylenes for 4 weeks (Toftgard et al. 1981). In rats exposed gestationally to mixed xylenes or *o*-xylene, a LOAEL of 500 ppm was identified for decreased fetal body weights in the absence of maternal toxicity (Bio/dynamics 1983). LOAELs for adult body weight effects were 1,000 ppm or higher (Tatrai et al. 1981). Increased deaths among squirrel monkeys and rats were noted following discontinuous intermediate-duration exposure to 780 ppm *o*-xylene (Jenkins et al. 1970), but no systemic effects were noted in rats or dogs exposed to 810 ppm mixed xylenes (Carpenter et al. 1975a).

Neurotoxicity is selected as the critical effect of intermediate-duration inhalation exposure to xylenes, since it occurs at the lowest effect levels and may persist after exposures cease. The study by Korsak et al. (1994) on *m*-xylene was selected as the principal study because it provides the lowest LOAEL (50 ppm) for the critical effect, neurotoxicity. In this study, groups of 12–24 male Wistar rats were exposed to 0, 50, or 100 ppm *m*-xylene, 6 hours/day, 5 days/week for 3 months. Before the study and at the end of each month of exposure, rats were evaluated for motor coordination using the rotarod performance test. The level of analgesia was tested at termination in the paw-lick response to hot-plate test at 54 °C. An increase in the failure rate on the rotarod performance test was concentration-related and statistically significant at 100 ppm, but not at 50 ppm. Mean latency of the paw-lick response was significantly reduced by 28% at 50 ppm compared to controls; this effect is considered minimal. The rat minimal LOAEL of 50 ppm was converted to a human equivalent following the EPA (1994) dosimetric equation for an extra-respiratory effect from an inhaled category 3 gas: $LOAEL_{HEC} = LOAEL_{ADJ} \times \lambda_a/\lambda_h$, where λ_a and λ_h are blood:air partition coefficients for rats and humans, respectively. Reported blood:air partition coefficients for *m*-xylene are 39.9 for male Wistar rats and 26.4 for humans (Sato and Nakajima 1979). Since the rat value is higher than the human, the default value of 1 was used for λ_a/λ_h (EPA 1994). The minimal $LOAEL_{HEC}$ is therefore 50 ppm. An uncertainty factor of 90 was applied to the minimal $LOAEL_{HEC}$ of 50 ppm (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability). The resulting MRL of 0.6 ppm is considered

to be protective to human health under intermediate-duration inhalation exposures to mixed xylenes or individual isomers.

- An MRL of 0.05 ppm has been derived for chronic-duration inhalation exposure (>1 year) to mixed xylenes.

The database for chronic-duration inhalation toxicity of xylene contains two studies that provide quantitative exposure information. An occupational study reported a LOAEL of 14 ppm for subjective respiratory and neurological effects in workers exposed to mixed xylenes for an average of 7 years (Uchida et al. 1993). This study examined 175 workers (107 men, 68 women) who were exposed to mixed xylenes (approximately 50% *m*-xylene, 30% *p*-xylene, and 15% *o*-xylene) during the manufacture of boots or rubber-coated wires. Subjects were selected between 1989 and 1991 from a group of 994 solvent-exposed workers who were supplied with diffusion samplers for one 8-hour workday; exposures were corroborated by measurements of xylene metabolites in urine. Subjects were evaluated for subjective symptoms in a questionnaire and also examined for objective parameters (serum biochemistry, hematology, and urinalysis). The final group of 175 exposed workers included those showing at least 70% of their solvent exposure to be from xylene and having completed all tests. The time-weighted average (TWA) (arithmetic mean) for xylenes was 21 ppm for an average of 7 years (geometric mean, 14 ppm); one man was exposed to 175 ppm. Xylenes represented at least 70% of the total solvent exposure, other chemicals being 3.4 ppm ethylbenzene (a typical component of commercial mixed xylene), 1.2 ppm toluene and rarely, n-hexane. No subjects were exposed to benzene, and the level of toluene exposure was low enough to be insignificant. The control group included 241 non-exposed workers (116 men, 125 women) in the same factories or elsewhere. Exposed workers showed a significant increase in the reporting of subjective symptoms including increased anxiety, forgetfulness, inability to concentrate, eye and nasal irritation, and sore throats. A concentration-relationship was reported for eye irritation, sore throat, and floating sensation, the last being indicative of central nervous system effect. There were no alterations in renal or hepatic serum parameters. This study has a few limitations: the lack of precise information on the duration of exposure and the lack of information as to how the workers used xylene on the job. Reliance on subjective symptoms might be considered a limitation of the study, except that these same symptoms were noted in the principal study for acute-duration inhalation exposure (Ernstgard et al. 2002).

The only other chronic-duration study was for rats exposed to 1,096 ppm *o*-xylene 8 hours/day, 7 days/week for 1 year (Tatrai et al. 1989). Hepatic changes included increases in liver weight and microsomal activity and proliferating endoplasmic reticulum, but no histopathological lesions. Body

weight was reduced by 11% in exposed rats. Calculation of a chronic-duration inhalation MRL from this rat study would not be appropriate since the study did not examine neurological effects, which are the most critical end points for inhalation exposure to xylene.

Respiratory and neurological effects are the co-critical effects of chronic-duration inhalation exposure to xylenes since they occurred at the lowest tested concentrations. The study of Uchida et al. (1993) was selected as the principal study for the chronic-duration inhalation MRL since it identified the lowest LOAEL of 14 ppm (geometric mean) for the co-critical effects. The geometric mean was used because it provides a better measure of central tendency than the arithmetic mean. The LOAEL of 14 ppm was divided by a total uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) and a modifying factor of 3 to account for lack of supporting studies evaluating the chronic neurotoxicity of xylene. The resulting MRL of 0.05 ppm is considered to be protective to human health under chronic-duration (>1 year) inhalation exposures to mixed xylenes or individual isomers.

Oral MRLs

- An MRL of 1 mg/kg/day has been derived for acute-duration oral exposure (≤14 days) to mixed xylenes.

Evaluation of the limited database for acute-duration oral exposure to xylenes suggests that neurotoxicity represents the most sensitive end point in laboratory animals; no quantitative human data are available. Effects observed in acute-duration oral studies in rats included altered visually evoked potentials following a single dose of 250 mg/kg *p*-xylene (Dyer et al. 1988), reduced pulmonary microsomal activity following a single dose of 1,000 mg/kg/day *p*-xylene (Patel et al. 1978), and reduced body weight gain following repeated dosing with 1,000 mg/kg/day mixed xylenes or 2,000 mg/kg/day *o*- or *p*-xylene (Condie et al. 1988; NTP 1986). Repeated gavage dosing with *p*-xylene, but not *m*- or *o*-xylene, at 900 mg/kg/day, 5 days/week for 2 weeks, resulted in significant loss of cochlear hair cells associated with hearing at midrange frequencies (10–25 kHz) (Gagnaire and Langlais 2005). Repeated exposure to mixed xylenes at 2,000 mg/kg/day, resulted in impaired respiration (shallow and/or labored breathing) in rats and mice and increased mortality (NTP 1986). At 2,060 mg/kg/day, developmental toxicity (cleft palate) was observed in mice gestationally exposed to mixed xylenes (Marks et al. 1982). Serious neurological effects (coma, incoordination, prostration, decreased hindleg movement) were observed in rats that received single oral gavage doses of ≥4,000 mg/kg mixed xylenes (Muralidhara and Krishnakumari 1980; NTP 1986).

Neurotoxicity was selected as the critical effect for acute-duration oral exposure to xylene because it was observed at the lowest exposure level and demonstrated dose-related increases in severity. The study by Dyer et al. (1988) was selected as the principal study, since it provides the lowest LOAEL for the critical effect. In this study, male Long-Evans rats with electrodes implanted for recording of brain visual potentials were dosed orally in two different experiments. In the first experiment, groups of 10–11 rats received single doses of *p*-xylene at doses of 0, 500, 1,000, or 2,000 mg/kg in corn oil and were tested 75 minutes later for potentials evoked in response to a single flash in 128 trials. The latencies and amplitudes of the P1, N1, P2, N2, P3, and N3 waveforms were determined. Based on the results of this experiment, groups of 14–16 rats were dosed at 0, 125, or 250 mg/kg and tested 45 minutes later to record the N3 waveform. Forty-five minutes after administration of 250 mg/kg, the amplitude of the N3 peak was decreased by 47% (statistically significant). At higher doses (500, 1,000, and 2,000 mg/kg), the N3 peak was not observed until 75 minutes after dosing. Thus, 125 mg/kg is a NOAEL and 250 mg/kg/day is a LOAEL for suppression of visual evoked brain potentials in rats exposed once to *p*-xylene. An uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) was applied to the NOAEL. The resulting MRL of 1 mg/kg/day is considered to be protective to human health under acute-duration oral exposures to mixed xylenes or individual isomers.

- An MRL of 0.4 mg/kg/day has been derived for intermediate-duration oral exposure (≥14 days to 1 year) to mixed xylenes.

The intermediate-duration oral toxicity of xylenes has been investigated in several oral gavage bioassays in rodents exposed to mixed xylene (Condie et al. 1988; NTP 1986), *m*-xylene (Elovaara et al. 1989; Wolfe 1988a), and *p*-xylene (Wolfe 1988b). The observed effects include increases in minimal chronic nephropathy and 17–37% increases in relative hepatic weight in rats exposed to ≥750 mg/kg/day mixed xylene (Condie et al. 1988); 27–46.3% increases in serum transaminase levels in rats exposed to 750–1,500 mg/kg/day mixed or *m*-xylene (Condie et al. 1988; Elovaara et al. 1989; Wolfe 1988a); reduced levels of cytochrome P-450 in the lung in rats exposed to 800 mg/kg *m*-xylene (Elovaara et al. 1989); 15–25% decreases in body weight gain in rats exposed to 800–1,000 mg/kg/day mixed, *m*-, or *p*-xylene (NTP 1986; Wolfe 1988a, 1988b); hyperactivity or increased aggressiveness subsequent to dosing with mixed xylene in rats at 1,500 mg/kg/day or mice at 1,000 mg/kg/day (Condie et al. 1988; NTP 1986); and 11% increased relative spleen weight, 16% increased relative kidney weight, and increased hematological effects (mild polycythemia and leukocytosis) in female rats dosed with 1,500 mg/kg/day mixed xylene (Condie et al. 1988). No hepatic effects were noted in F344 rats given mixed xylene at doses as high as 1,000 mg/kg/day or B6C3F$_1$ mice dosed at ≤2,000 mg/kg/day 5 days/week for 13 weeks (NTP 1986) or in Sprague-Dawley rats dosed with *p*-xylene at 800 mg/kg/day for 90 days (Wolfe 1988b). No renal effects

were observed in rats or mice exposed to mixed xylene in studies by NTP (1986) or in rats exposed to *m*- or *p*-xylene (Wolfe 1988a, 1988b). The lack of hepatic or renal effects in some studies may be related to strain differences, exposure differences (discontinuous vs. continuous), or isomer specificities.

A limitation of standard intermediate-duration oral bioassays for xylene is that no testing was conducted for sensitive neurological effects. The only overt neurological effect of long-term exposure to xylene was hyperactivity noted in all male and female $B6C3F_1$ mice (50/sex/group) immediately after oral gavage dosing 5 days/week with 1,000 mg/kg (710 mg/kg/day duration adjusted) mixed xylene (9.1% *o*-, 60.2% *m*-, and 13.6% *p*-xylene plus 17% ethylbenzene) in corn oil beginning at week 4 of the 103-week NTP (1986) bioassay; hyperactivity was not observed at 500 mg/kg (360 mg/kg/day, duration-adjusted). Survival was not significantly affected by treatment with xylene during the first year. Neurotoxicity (hyperactivity) observed during the first year of that study (weeks 4–51) is selected as the critical effect of intermediate-duration exposure because it was observed at the lowest LOAEL (710 mg/kg/day, adjusted for intermittent exposure). Selection of neurotoxicity as the critical effect is consistent with other MRLs for xylene. The NTP (1986) study is selected as the principal study for intermediate-duration exposure to xylene because it provides the lowest adverse effect level, a LOAEL of 710 mg/kg/day, and a NOAEL of 500 mg/kg (360 mg/kg/day, duration-adjusted) for the critical effect. A total uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability) and a modifying factor of 10 (for the lack of testing for sensitive neurological end points and lack of developmental and multi-generational data) were applied to the duration-adjusted NOAEL. The resulting MRL of 0.4 mg/kg/day is considered to be protective to human health under intermediate-duration oral exposures to mixed xylenes or individual isomers.

- An MRL of 0.2 mg/kg/day has been derived for chronic-duration oral exposure (≥1 year) to mixed xylenes.

The available animal studies involving chronic-duration oral exposure to xylenes do not clearly identify toxic effects other than 5–8% decreases in body weight gain (not biologically significant) and unexplained reduced survival in male F344 rats at 500 mg/kg (NTP 1986) and transient hyperactivity associated with gavage administration in male and female $B6C3F_1$ mice at 1,000 mg/kg/day (NTP 1986). These studies are standard cancer/toxicology bioassays involving administration of mixed xylenes (13.6% *p*-xylene, 60.2% *m*-xylene, 9.1% *o*-xylene, and 17% ethylbenzene) by gavage in corn oil to groups of 50 animals/sex, 5 days/week for 103 weeks (NTP 1986). Animals were examined twice daily and clinical signs were recorded daily for the first 16 months, then once a month thereafter. Body weights were recorded weekly for the first 12 weeks and then once every 4 weeks thereafter. At termination, all

animals were subjected to gross necropsy and histological examinations. Comprehensive histological examination of major tissues and organs revealed no exposure-related increased incidences of pathological lesions in rats or mice.

In the absence of data associating health effects in humans with chronic-duration oral exposure to xylenes, the animal bioassays provide a minimal basis for deriving an MRL for humans chronically exposed to xylenes. A NOAEL of 500 mg/kg was identified for hyperactivity in mice, but this was not selected as the basis for the chronic-duration MRL because that dose decreased survival in male rats. Therefore, the rat NOAEL of 250 mg/kg was selected as the basis of the MRL. The NOAEL was first adjusted for discontinuous exposure (5 days/7 days), resulting in a duration-adjusted NOAEL of 179 mg/kg/day. An uncertainty factor of 100 (10 for extrapolation between animals and humans and 10 for human variability) and a modifying factor of 10 were applied to the duration-adjusted NOAEL to account for the lack of testing for sensitive neurological end points (the most sensitive effects in inhalation studies and acute oral studies) and lack of developmental and multi-generational data. The resulting chronic-duration oral MRL of 0.2 mg/kg/day is considered to be protective to human health under chronic-duration (>1 year) oral exposures to mixed xylenes or individual isomers.