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TOXICOLOGICAL REVIEW

OF

XYLENES

(CAS No. 1330-20-7)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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Washington, D.C.

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CONTENTS — TOXICOLOGICAL REVIEW for XYLENES (CAS No. 1330-20-7)

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to xylenes. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of xylenes.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to the IRIS Hotline at 202-566-1676.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Managers/Authors

Chemical Managers

Michael W. Broder, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Lynn Flowers, Ph.D., DABT
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Authors

Lynn Flowers, Ph.D, DABT
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Michael W. Broder, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Peter McClure, Ph.D., DABT
Syracuse Research Corporation
North Syracuse, NY

Mark Osier, Ph.D.
Syracuse Research Corporation
North Syracuse, NY

Claudia Troxel, Ph.D.
Oak Ridge National Laboratory
Oak Ridge, TN

Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

Internal EPA Reviewers

Elaina Kenyon, Ph.D.
National Health and Environmental Effects Research Laboratory

Bruce Rodan, MD
National Center for Environmental Assessment

Kevin Crofton, Ph.D.
National Health and Environmental Effects Research Laboratory

External Peer Reviewers

Robert A. Howd, Ph.D.
California Environmental Protection Agency
Sacramento, CA

Patricia McGinnis, Ph.D.
Syracuse Research Corporation
Ft. Washington, PA

Bonnie R. Stern, Ph.D., M.P.H.
BRC Associates
Washington, DC

Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds generally exist for noncancer effects. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or system effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for xylenes has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S.

EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The initial literature search strategy employed for this compound was based on the CASRN and the common name for individual isomers as well as the mixture. The large number of citations for the CASRN and common name necessitated a refinement of the search strategy that involved identifying older research from reviews and chemical assessments combined with a thorough review of the recent publications. The following data bases were searched: TOXLINE (all subfiles), MEDLINE, CANCERLIT, TOXNET [HSDB, IRIS, CCRIS, EMIC (1991-2002), and GENE-TOX], and RTECS, in conjunction with a comprehensive DIALOG search. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Commercial or mixed xylenes are composed of three isomers: *meta*-xylene (m-xylene), *ortho*-xylene (o-xylene), and *para*-xylene (p-xylene), of which the m-isomer usually predominates (44–70% of the mixture) (Fishbein, 1988; ATSDR, 1995). The exact composition of the isomers commonly depends on the source. Ethylbenzene is commonly present in mixed xylenes; in fact, the technical product contains approximately 40% m-xylene and approximately 20% each of o-xylene, p-xylene, and ethylbenzene (Fishbein, 1988). Thus, most of the environmental and occupational exposures and toxicological studies are conducted on this mixture of xylenes containing ethylbenzene. Other minor contaminants of xylenes include toluene and C_9 aromatic fractions. Some physicochemical data for xylenes are shown in Table 1.

Mixed xylenes are used in the production of the individual isomers or ethylbenzene, as a solvent, in paints and coatings, or as a blend in gasoline (Fishbein, 1988; ATSDR, 1995). The annual U.S. manufacturers' production capacity of mixed xylenes has been estimated to be 13.1 billion pounds, based on maximum plant production volumes (ATSDR, 1995). The annual U.S. production of xylenes for 1990-1991 has been estimated at about 6–12 billion pounds for mixed xylenes, 900 million pounds for o-xylene, 5–8 billion pounds for p-xylene, and 169 million pounds for m-xylene (ATSDR, 1995). The nonconfidential U.S. aggregate production volumes for 1998, based on industry submissions to U.S. EPA, are: mixed xylenes >1 billion pounds, m-xylene >100–500 million pounds, o-xylene >1 billion pounds, and p-xylene >1 billion pounds (U.S. EPA, 1998c).

Table 1. Physicochemical data for xylenes

Parameter	Value	Reference
Synonyms	dimethylbenzene (1,2-; 1,3-; or 1,4-); xylol (mixture), m-, o-, or p-xylene (isomers); methyltoluene	Budavari et al. (1996); ACGIH (1991)
CAS registry no.	1330-20-7 mixture 108-38-3 m-isomer 95-47-6 o-isomer 106-42-3 p-isomer	
Chemical formula	C_8H_{10}	Budavari et al. (1996)
Molecular weight	106.17	Budavari et al. (1996)
Physical state	liquid	Budavari et al. (1996)
Vapor pressure at 20°C	6–16 mmHg mixture 5–6.5 mmHg individual isomers	ATSDR (1995)
Density	0.864 g/cm^3 mixture 0.8642 g/cm^3 m-isomer 0.8801 g/cm^3 o-isomer 0.8611 g/cm^3 p-isomer	ATSDR (1995)
Melting point	No data for mixture –47.4°C m-isomer –25°C o-isomer 13–14°C p-isomer	Budavari et al. (1996)
Boiling point	137–140°C mixture 139°C m-isomer 144.4°C o-isomer 138.37°C p-isomer	Budavari et al. (1996); ATSDR (1995)
Solubility in water	130 mg/L mixture 134–146 mg/L m-isomer 178–213 mg/L o-isomer 185–198 mg/L p-isomer	ATSDR (1995)
Log K_{OW}	3.12–3.20 mixture 3.20 m-isomer 3.12, 2.77 o-isomer 3.15 p-isomer	ATSDR (1995)
Conversion factors in air	1 ppm = 4.34 mg/m^3 1 mg/m^3 = 0.23 ppm	NRC (1984)
Odor threshold in air (absolute)	1.0 ppm mixture 3.7 ppm m-isomer 0.08–0.17 ppm o-isomer 0.47 ppm p-isomer	ATSDR (1995)

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

The available studies indicate that xylenes are rapidly absorbed following both inhalation and oral exposure. Following absorption, considerable metabolism occurs, with the liver being the primary site of metabolism. Xylenes are distributed throughout the body, but show the greatest affinity for lipid-rich tissues such as adipose tissue or the brain. Elimination is rapid and occurs primarily in the urine, with the predominant form being the glycine conjugate of methylbenzoic acid (methylhippuric acid). In humans exposed by inhalation, the loss of xylene from the blood has been shown to follow biphasic, first-order kinetics with half-lives of about 0.5–1 hour and 20–30 hours.

3.1. ABSORPTION

The rapid absorption of xylenes into the body is demonstrated by numerous experimental observations. Sato and Nakajima (1979) conducted studies to determine the partition coefficients of each of the three isomers of xylene. The blood/air and oil/blood partition coefficients are useful as surrogates for assessing the relative solubility of the respective chemical for movement into the blood from inhaled air and movement from the blood into tissue. The study authors employed olive oil and blood (source not provided). The blood/air partition coefficient for the three isomers ranged from 26.4 to 37.6, and the oil/blood partition coefficient ranged from 98 to 146. These data indicate that xylene entering the body would be readily absorbed into the blood and would be expected to move from the blood into tissues in which neutral lipids predominate, such as adipose tissue. The low water solubility of xylenes suggests that xylene would move into portions of tissues that have a high lipid fraction. Accordingly, low water solubility may also serve as a boundary by impeding movement of xylenes from the gaseous phase into tissues that contain a liquid coating, most notably pulmonary tissue.

Several studies have been conducted on the uptake of xylenes by inhalation. Ogata et al. (1970) exposed human volunteers to either m- or p-xylene in an exposure chamber for either 3 hours or 7 hours with a 1-hour break at midday. The authors provided no information on the level of activity of the subjects (i.e., whether the subjects were sedentary or physically active). During the last 2 hours of exposure the researchers determined that the retention of xylene in lungs was 87%. This value is considerably higher than values found in other studies and may reflect the quality of instrumentation used in this study. The authors noted that their methods were not state of the art, but they were used to maintain consistency with earlier studies.

Sedivec and Flek (1976a) made direct measurements of the level of absorption of xylenes by subjecting volunteers to 200 or 400 mg/m^3 (46 or 92 ppm) of either individual isomers or mixtures of the three isomers of xylenes vapor for 8 hours without interruption and measuring the difference in the concentration of xylenes in the inspired air relative to the amount expired. The amount of the individual isomers absorbed over time was consistent for all three isomers and ranged from 62.4 to 64.2% of the inhaled volume, reflecting a high solubility of xylenes in blood.

Riihimäki and Savolainen (1980) conducted studies on human subjects both at rest and during exercise to measure the kinetics resulting from exposure to mixed xylenes. Healthy male subjects were exposed to xylene for 5 days, 6 hours per day with a 1-hour break at midday and then for an additional 1 to 3 days after a 2-day weekend break. The exposure scenarios included either constant exposure to 100 or 200 ppm or fluctuating exposure with peaks of 200 or 400 ppm that lasted for 10 minutes. The subjects were either sedentary or exercising on a stationary bike for short periods of time.

Regardless of the exposure scenario (constant or fluctuating or with different xylene concentrations), retention consistently remained around 60% (i.e., 60% of the inhaled xylene was retained in the blood and 40% was expired). The results indicate that partitioning of xylene between the tissues and the air occurs, but it is limited by the solubility in the tissue lipids and the rate of passive diffusion through the matrix. Overall, the lowest uptake rate was noted with 100 ppm exposure during sedentary conditions (22 μmol/min) and the highest uptake was seen with the fluctuating concentrations in which the peaks reached 400 ppm during exercise (266 μmol/min). Given the constant retention values, the two factors that appeared to control the total uptake of xylenes were the ambient concentration of xylene and ventilation rates of the subjects.

Astrand et al. (1978) subjected volunteers to vapors of mixed xylenes for four periods of 30 minutes each. Volunteers in the first group were exposed to 870 mg/m^3 (200 ppm) vapors for 30 minutes at rest and 90 minutes during light exercise that required 30% of the subjects' maximal oxygen uptake. The second group was exposed to 435 mg/m^3 (100 ppm) with no activity for 30 minutes followed by three successive 30-minute step intervals of increasingly demanding workload that required up to 50% of the subjects' maximal oxygen uptake. The authors monitored the amount of xylene in the inspired and expired air and in the arterial and venous blood to measure the uptake of xylenes into the blood.

The amount of xylenes taken up in the group with continuous light exercise was constant over the 2-hour period, with about 65% of the inspired xylenes absorbed by the body. With regard to increasing workload, retention started at about 65% but dropped to 50% at the higher workloads and the corresponding increase with ventilation rate. Over the 2-hour period, the volunteers subjected to 870 mg/m^3 and the light workload absorbed 1.4 g of the mixed xylenes and the group exposed to 435 mg/m^3 and the increasingly demanding workload absorbed about 1.0 g. The rate of absorption of xylenes in the first group remained constant over the final 90 minutes, indicating that for a 2-hour exposure, equilibrium between the blood and air had not been reached. The fact that the constant absorption rate was 64–65% for the first group and the rate never dropped below 50% for the second group indicates both the high affinity of xylene for blood and the rapid metabolism of xylene in the body. The lower retention observed in the second group reflects the ventilation rate. However, the authors estimated that the two groups inhaled a total of 2.2 and 1.7 g xylene and retained 1.4 and 1.0 g, respectively.

Senczuk and Orlowski (1978) conducted three measurements each on 10 healthy volunteers (5 men and 5 women) between the ages of 17 and 33 years (for a total of 30 experiments). The individuals were exposed to m-xylene vapor in an inhalation chamber at three concentrations (100, 300, and 600 mg/m^3) for 8 hours with two half-hour breaks. The

investigators monitored the concentration of m-xylene in the vapor and expired air. Xylene retention and urinary levels of m-methylhippuric acid were also measured. The investigators found that the retention of m-xylene in the lung varied with the concentration of m-xylene and duration of exposure. At 300 mg/m^3, retention decreased from 83% at the start of the study to 67% at the end of the exposure period (mean, 75%). At 600 mg/m^3, retention decreased from 78% at the start of the study to 65% (mean, 71%) at the end of the exposure period. At 100 mg/m^3 there was relatively little change in retention rate: 87% at the start and 84% at the end of exposure. The time periods at which the retention measurements occurred were not specified. The total amount of m-xylene absorbed was 272, 724, and 1359 mg for women and 342, 909, and 1712 mg for men at exposures of 100, 300, and 600 mg/m^3, respectively.

David et al. (1979) conducted comparative studies on the uptake and metabolism of m-xylene by inhalation in humans and rats. The objective of the study was to evaluate the effects of induction of metabolizing enzymes on the ability of the body to clear m-xylenes at different concentrations. The human component involved five healthy volunteers between the ages of 46 and 55 years who, during one stage of the study, were exposed to m-xylene without pretreatment with phenobarbital and during another stage with the equivalent of 2 mg/kg-day phenobarbital for 11 days prior to treatment with m-xylene. The subjects were exposed to 400 mg/m^3 of m-xylene for 8 hours in a chamber (no description of the level of activity or ventilation rates of the subjects was provided). The retention rates for the controls and phenobarbital-treated subjects averaged 58% and 59%, respectively, over the course of exposure with no difference between the morning and afternoon exposure periods, indicating that equilibrium is not reached at these exposure concentrations and times.

Animal studies (Turkall et al., 1992; Kaneko et al., 1995) have demonstrated rapid and extensive uptake via the oral route through the detection of parent material in blood or metabolites in the urine.

Following oral administration of 0.081 or 0.81 mmol/kg (8.6 or 86.4 mg/kg) m-xylene in corn oil to male Wistar rats, Kaneko et al. (1995) found that blood concentrations of m-xylene rapidly increased within 4–6 hours and declined thereafter, indicating rapid absorption by the gastrointestinal tract. Peak concentrations were about 2.5 μM for the low dose and about 55 μM for the high dose. Cumulative amounts of the metabolite m-methylhippuric acid in urine increased through about 12 hours after dose administration and reached an apparent plateau thereafter. Final cumulative amounts of m-methylhippuric acid in the urine (through 48 hours) were about 22 μmoles for the low dose and 160 μmoles for the high dose. The authors noted that because m-xylene is subject to metabolism in the liver and has a high blood/air partition coefficient, more than 90% of the dose would be expected to be excreted as metabolites in the urine and less than 10% excreted unchanged in expired air.

To examine the influence of soil adsorption on oral absorption, distribution, and elimination of m-xylene, groups of six male or six female Sprague-Dawley rats were orally administered doses of ^{14}C-ring-labeled m-xylene in gum acacia alone or with samples of a sandy soil or a clay soil (Turkall et al., 1992). Each dose contained 150 μL m-xylene (about 130 mg,

assuming a density of 0.864 g/mL). Radioactivity was measured in samples of blood (plasma), feces, urine, and expired air collected at several intervals up to 48 hours after dosing. Five rats of each sex were sacrificed at 24 hours after dosing, and levels of radioactivity were determined in fat, stomach, pancreas, and skin.

Absorption from the gastrointestinal tract was rapid for both sexes in all treatment groups; maximum peak plasma concentrations of radioactivity occurred within 20 minutes after dosing. From the time course of plasma radioactivity, absorption and elimination half-times were calculated. In the xylene-alone treatment groups, the mean absorption half-time in females (0.31 hours) was statistically significantly shorter than that in male rats (0.64 hours), whereas the elimination half-time was longer in females (11.42 hours) than in males (6.77 hours). These results suggest that absorption may be faster and elimination slower in female rats than in male rats. Both soil treatments resulted in statistically significantly increased absorption half-times over xylene alone in female rats, but no effect of soil treatment on this variable was apparent in male rats. Female rats treated with the sandy soil showed a significantly increased area under the curve (AUC) for plasma-concentration-time as compared with AUCs for other female rat groups, indicating that this soil may have increased bioavailability of xylene in females.

No statistically significant differences were observed between males and females in the amount of radioactivity in the urine collected over 48 hours. Mean levels of radioactivity expressed as a percentage of the administered dose were 73.7 ± 4.9, 73.2 ± 16.3, and 78.2 ± 0.6 for the xylene alone, xylene and sandy soil, and xylene and clay soil female groups, respectively, and 96.2 ± 8.8, 83.3 ± 2.9, and 79.4 ± 3.3 for the male groups. These results suggest extensive absorption by the gastrointestinal tract in both sexes ranging from about 74 to 96% of the administered dose of m-xylene. Radioactivity as parent compound in expired air was a secondary route of elimination, representing about 14–22% of the administered dose in females and 9–20% in males. Tissue concentrations of radioactivity at 24 hours were highest in the fat for both sexes in all treatments.

3.2. DISTRIBUTION

The K_{ow} of xylene indicates that xylene is expected to partition primarily into tissues containing a higher proportion of neutral lipids, such as adipose, liver, and brain tissue. Kumarathasan et al. (1998) conducted studies with organs and blood from Sprague-Dawley rats to determine tissue/blood partition coefficients (K_d) for brain, muscle, kidney, liver, and fat. The rats were sacrificed and the organs of interest were removed. The tissues were trimmed to remove extraneous tissue and to achieve uniform size, after which they were spiked with varying concentrations of a mixture of ethylbenzene and o-, m-, and p-xylenes. Following treatment, the tissues were placed into vials that were sealed and allowed to equilibrate for either 1 or 2 days. The concentration of the ethylbenzene and individual isomers of xylene in the head space was determined by gas chromatography. The K_d for xylene in brain, muscle, and kidney were comparable and ranged from 1.5 to 3.7. The range of K_d includes ranges in the administered tissue dose for the three isomers. The K_d for liver was slightly higher, ranging from 3.2 to 5.7. The K_d for fat was the highest in all tissues tested, with values ranging from 37 to 67; one value

of 26 appeared to be an outlier. There were no differences across administered tissue doses or between isomers.

In their study on the uptake and distribution of ethylbenzene and xylenes, Riihimäki and Savolainen (1980) found that 10–20% of the xylene was distributed to the adipose tissue. Adipose has the highest concentration of neutral fat and the highest affinity for xylene of all tissues. Therefore, once sequestered in adipose tissue, xylene is expected to have the lowest rate of metabolism, the slowest movement to blood, and the longest persistence in the body. The concentration of xylene in gluteal subcutaneous fat was about 10-fold higher than in venous blood following the last day of exposure (5 days exposure + weekend without exposure + 1 day of exposure).

Astrand et al. (1978), in their study discussed above, found that following rapid uptake of xylene vapors, concentrations increased in the arterial and venous blood. An initially rapid but persistent loss of xylenes from the blood followed cessation of exposure. Despite the rapid absorption of xylenes, the amount found in the blood generally constituted 2–3% of the total xylenes absorbed. The authors postulate that these factors reflect the high lipid solubility of xylenes, resulting in the distribution and storage of xylenes, in various tissues. As xylenes are lost from the blood, residual xylene stored in tissue is eluted into the blood. The higher affinity of xylene for lipid indicates that the loss of xylene from the tissue is a slow process.

In a study similar to that of Astrand et al. (1978), Engstrom and Bjurstrom (1978) exposed volunteers to either 870 mg/m^3 of xylene vapors during a 30-minute resting period followed by light exercise or 435 mg/m^3 of xylene vapors during a 30-minute resting period followed by 90 minutes of increasingly strenuous exercise. Adipose tissue was sampled for xylenes from volunteers at 0.5, 2, 4, and 20–24 hours following conclusion of exposure. The amount of solvent stored in the body was highly correlated with the amount of body fat. A direct correlation was found between the amount of xylene taken up and body fat when the two exposure groups were analyzed together. The mean of the high-exposure group was higher than that seen with the low-exposure group during the first 4 hours of the study, despite the higher rate of ventilation in the lower-exposure group. However, at the 20–24 hour sampling, the amount of xylene in the adipose tissue of the low-exposure group was slightly, but not significantly (p>0.10), higher than the high-exposure group. The concentration of xylenes in the adipose tissue was comparable or higher at the 20–24 hour sampling than at the 4-hour sampling. These data reflect a high absorption of xylenes from the blood into the tissue extending beyond the exposure period.

Additional information on the distribution of xylenes in the body is available from rodent studies. Kumarathasan et al. (1997) exposed Sprague-Dawley rats to 1100 ppm m-xylene vapor in an inhalation chamber for 2 hours. After exposure, the rats were removed from the inhalation chamber, treated with anaesthetic, and returned to the chamber to avoid loss of xylene. After the anaesthetic had taken effect, a blood sample was taken from the aorta, the animals were sacrificed, and the kidney, liver, brain, and fat were harvested. Head-space samples were taken and analyzed by gas chromatography for m-xylene after 1 day for fat and 2 days for the other

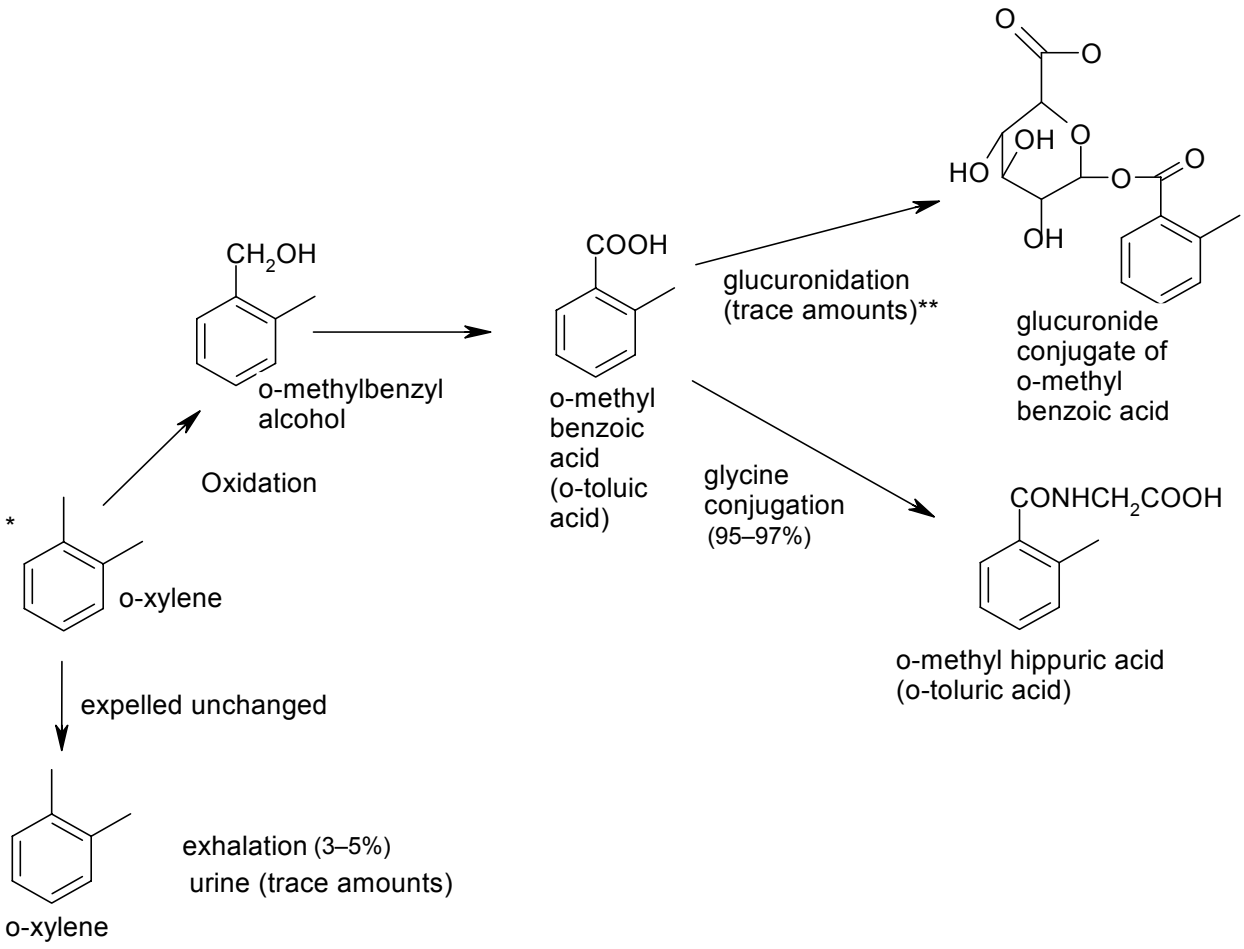
tissues. On a per-gram-of-tissue basis, brain and kidney had the lowest level of m-xylene, followed by liver; fat contained a considerably higher level than the other tissues.

Carlsson (1981) determined tissue concentrations of m-xylene in male rats following inhalation exposure to 48 ppm radiolabeled p-xylene for 1, 2, 4, or 8 hours. The greatest concentration of xylene equivalents (combined concentration of xylene and its metabolites) was found in the kidneys immediately following the 4-hour exposure (1080 ± 366 nmol/g tissue), with the next highest concentration found in the subcutaneous fat. The concentration in the subcutaneous fat continued to increase, reaching peak concentration following the 8-hour exposure (270 ± 7 nmol/g tissue). For the remaining tissues, the relative m-xylene concentrations were ischiadic nerve > blood = liver ≥ lungs > cerebrum = cerebellum = muscles = spleen. Concentrations of xylene-equivalents in the cerebellum, cerebrum, muscles, spleen, and lungs paralleled the concentrations of xylenes in the arterial blood throughout the entire exposure period. The distribution of xylenes in tissues parallels that seen in Kumarathasan et al. (1998).

Bergman (1983) investigated the distribution of radiolabeled m-xylene in mice using low-temperature, whole-body autoradiography and found high radioactivity levels in the body fat, bone marrow, white matter of the brain, spinal cord, spinal nerves, liver, and kidney immediately following inhalation. High levels of metabolites were present in the blood, liver, lung, kidney, and adrenal medulla; only the parent compound was found in the body fat, bone marrow, and white matter of the brain. High levels of metabolites were observed in the kidneys up to 4 hours, in the liver up to 2 hours, in the bile from 2 to 8 hours, in the nasal mucosa and bronchi from 2 to 24 hours, and in the adrenal medulla immediately after exposure (with no detectable levels by 30 minutes). No radioactivity was detected in the body by 48 hours after exposure. Additionally, the author reported that no metabolites of m-xylene were firmly bound in the tissues.

3.3. METABOLISM

Proposed metabolic pathways for o-xylene are shown in Figure 1 as a model for all xylene isomers. The principal metabolic fate involves oxidation of one of the methyl groups to a methylbenzoic acid derivative via methylbenzyl alcohol and methylbenzaldehyde intermediates (only methylbenzyl alcohol is shown in Figure 1). The methylbenzoic acid derivative is mostly conjugated to glycine, producing methylhippuric acid derivatives that can be excreted in urine. Conjugation to glucuronic acid is a minor pathway. Oxidation of the benzene ring to produce xylenols (i.e., dimethylphenols) is expected to be a negligible metabolic pathway, based on



* o-xylene used as a model for all isomers of xylene
** significant production of glucuronic derivative under conditions of high levels of administration

Figure 1. Metabolic pathways for xylenes.

Source: Adapted from Ogata et al., 1970; Riihimaki and Savolainen, 1980; Riihimaki, 1979; Bray et al., 1949; Sedivec and Flek, 1976a,b; Ogata et al., 1980; Carlsson, 1981; Senszuk and Orlowski, 1978; David et al., 1979.

analysis of urinary metabolites, and is not included in Figure 1. The liver is expected to be the principal site of metabolism for xylenes.

In the study described above, Ogata et al. (1970) exposed human volunteers to differing concentrations of either m- or p-xylene for varying periods of time. The data demonstrated a linear relationship between exposure to xylenes expressed either in concentration or amount of methylhippuric acid excreted in the urine over an 18-hour period. These data also demonstrated that the rates of excretion for the two isomers were similar. The authors used these data to demonstrate that methylhippuric acid could serve as a marker of exposure to xylene.

Riihimäki and Savolainen (1980), in their study of inhalation exposure to xylene by human subjects under sedentary and physically active conditions, found that 95% of the eliminated xylene was in the form of methylhippuric acid, with the remainder lost as unmetabolized xylene in expired air. No deposition sites, such as lipid-rich tissues, were studied.

Riihimäki (1979) evaluated the metabolism and excretion of xylene and toluene derivatives in humans. A volunteer was administered a single dose of 7.4 mmole m-methylbenzoic acid or 7.8 mmole m-methylhippuric acid. Urine was analyzed for 30 hours following administration for the presence of metabolites. All of the administered xylene derivatives appeared in urine as methylhippuric acid, indicating that, under the conditions of this study, once xylene has been oxidized to methylbenzoic acid, the only route of metabolism was as the glycine conjugate. The relevance of metabolism to the rate of excretion is discussed below.

Bray et al. (1949) intubated and fed rabbits either o-, m-, or p-xylene or the corresponding toluamide or toluic acid (methylbenzoic acid) and analyzed for metabolites in the urine. The authors relied on rudimentary colorimetric methods. Higher levels of the glucuronide metabolite were found in the urine of rabbits that were force-fed the methylbenzoic acid metabolite and relatively small amounts of the glucuronide following force-feeding with xylene. From these data, the authors concluded that xylenes are metabolized first to methylbenzoic acid, which is subsequently conjugated with glycine; the rate-limiting step of this process is the conversion of xylene to methylbenzoic acid. In the absence of sufficient amounts of glycine, as with the bolus administration of methylbenzoic acid, the acid reacts with other possible reactants. The authors noted that there was a difference in the metabolism of o-xylene compared with the other two isomers that may have been related to unexplained differences in the excretion of the chemical. Nonetheless, with bolus administration of the xylenes, most of the chemical was excreted as either the acid or glycine conjugate (i.e., methylhippuric acid).

Sedivec and Fleck (1976a) measured the production of conjugates of methylbenzoic acid in the urine of subjects exposed to 200 and 400 mg/m^3 of individual isomers of xylene vapor. In contrast with the findings of Bray et al. (1949), all of the methylbenzoic acid derivatives in the urine were in the form of glycine conjugates (hippuric acid and methylhippuric acid), with no evidence of glucuronic conjugates. The lack of glucuronide conjugation was attributed to the method of dosing: Bray et al. used an oral bolus administration of 0.6 g/kg as compared with their estimated administration by Sedivec and Fleck of 0.019 g/kg by the inhalation route. The metabolic differences may be species-specific (rabbits and humans), with dosing as the decisive factor. In addition, xylenols were formed at considerably lower concentrations than were the methylhippuric acids.

A similar finding was reported by Ogata et al. (1980). The researchers conducted studies under three different scenarios: intraperitoneal (i.p.) injection of xylenes to rats (11.3 mmol/kg), oral administration in human volunteers (0.368 and 0.736 mmol/kg), and inhalation exposure by human volunteers (138 ppm for 3 hours) followed by analysis of urine over time. The results demonstrated that at the highest doses (e.g., rats at 11.3 mmol/kg i.p. xylene), the relative amount of methylbenzoic acid glucuronide was the highest and the glycine conjugate, methylhippuric acid, was the lowest. The inhalation exposure demonstrated the highest relative amounts of methylhippuric acid and almost no detectable methylbenzoic acid glucuronide. One explanation for these results may be differences in the toxicokinetics between species, or they could reflect differences arising from routes of administration, as the rats received i.p. injections, which may have led to saturation of enzyme systems or depletion of glycine stores. The lowest dose was through inhalation, which occurred over 3 hours, as compared with bolus administration by i.p. or oral ingestion. The administration by i.p. injection of o-xylene in rats reflected the highest amount and proportion of unmetabolized xylene in the urine of the three scenarios. Although these data may reflect species specificity, the differences in the dosing levels and routes of administration appear to place different burdens on the respective enzyme systems.

In a study noted above (Senczuk and Orlowski, 1978), five male and five female volunteers were exposed to three concentrations of m-xylene vapor. The study was designed to develop a method for quantifying the exposure of individuals to m-xylene using methylhippuric acid as an indicator; therefore, no measurement was made of other metabolites of m-xylene nor was there a measurement of the amount of xylene expired following cessation of exposure. The study found that about 90% of the xylene absorbed was converted to methylhippuric acid.

Astrand et al. (1978) subjected volunteers to concentrations of mixed xylene vapor and measured the persistence of xylene in the body. One procedure involved exposure to xylenes at 870 mg/m^3 while resting for 30 minutes followed by 90 minutes of light exercise and the other exposure to 435 mg/m^3 while resting followed by 90 minutes of increasingly demanding exercise. The authors tracked the amount of xylenes in both venous and arterial blood and the amount expelled through the lungs. They found that following cessation of exposure the amount of xylenes in the arterial and venous blood decreased rapidly. A total of 5% and 4% of the xylenes absorbed during exposure while resting followed by light exercise and resting with variable exercise exposures, respectively, were exhaled as unmetabolized xylenes. The remainder was assumed to be excreted in the urine.

The authors attributed the rapid removal of xylene from the blood to metabolism of the xylenes (although they did not measure the production of methylhippuric acid or the amount of xylene or metabolites in the urine). Despite the rapid initial removal of xylenes, the authors were able to detect the presence of xylenes 4 to 5 days following exposure. The presence of xylenes in

the blood most likely reflects their high solubility for the lipid component in tissues. As the xylenes in the blood decrease over time, the equilibrium shifts from movement from blood to tissues to movement out of the tissues and into the blood, albeit at a slower rate.

In the study mentioned above, Carlsson (1981) measured the distribution of xylene and its metabolites. During exposure, the highest proportions of the dose present as a metabolite were found in liver and blood; each contained 60–70% of the total xylene equivalent as metabolites (mostly methylhippuric acid). Subcutaneous fat had the lowest proportion (20%) of metabolized xylene. Three hours following cessation of exposure, the concentration of xylene equivalents in the form of metabolites increased from 50–67% to about 95% in the liver. However, in the muscle, the proportion of xylene equivalents as metabolites ranged from 61 to 71% during exposure, then subsequently decreased to 40% at 3 hours following exposure. In subcutaneous fat, the relative concentration of xylene equivalents in the metabolite form remained constant at about 20%.

Results from rat studies demonstrate that orally administered xylenes are subject to a first-pass metabolic effect that limits the amount of absorbed parent material reaching the general circulation (Kaneko et al., 1995). The results, however, do not identify oral dose levels that saturate hepatic metabolism and overwhelm the first-pass effect. Oral doses of 0.081 or 0.81 mmol/kg (8.6 and 86.4 mg/kg) m-xylene in corn oil were given to groups of five male Wistar rats that were pretreated for 3 days with 80 mg/kg/day phenobarbital in saline or with saline alone. Other groups of five rats with or without phenobarbital pretreatment were exposed by inhalation to 40 or 400 ppm m-xylene for 6 hours. At preselected intervals up to 6 hours after inhalation exposure and up to 12 hours after oral dose administration, blood samples were collected from the tail. Blood concentrations of m-xylene were measured by a syringe equilibration method. Phenobarbital pretreatment was administered to examine the effect of induction of hepatic CYP2B1 on the amounts of parent material in the blood. The AUCs of the plot of m-xylene blood concentrations versus time after exposure were taken as an index of the amount of parent material in the blood.

Pretreatment with phenobarbital did not influence the AUCs for rats exposed to the low air concentration, but it statistically significantly decreased the AUCs for rats exposed to the high concentration. At 40 ppm, AUCs (in units of μM x hour) were 15.3 ± 1.5 and 15.6 ± 1.2 for nonpretreated and pretreated rats; at 400 ppm, respective AUCs were 361 ± 58 and 208 ± 25. Pretreatment with phenobarbital statistically significantly decreased AUCs for rats exposed to either oral dose level when compared with nonpretreated rats. At 0.081 mmol/kg, AUCs were 14.3 ± 1.2 and 4.81 ± 0.23 for nonpretreated and pretreated rats; at 0.81 mg/kg, respective AUCs were 326 ± 29 and 28.6 ± 1.9. These results demonstrate that first-pass metabolism under an "induced" state can greatly influence the amount of orally administered m-xylene that reaches the blood, but they do not identify dose levels at which the first-pass effect is exceeded. For inhalation exposure, the results show that hepatic enzyme induction influences the amount of parent material in the blood only at a high air concentration (400 ppm) and not at a low concentration (40 ppm).

3.4. EXCRETION

Results from the study by Riihimäki (1979) (described above) indicate that the rate-limiting step for the excretion of xylene is the conjugation of methylbenzoic acid with glycine. A single volunteer was, at different times, administered methylbenzoic acid or its glycine conjugate, methylhippuric acid. Urine was collected over 30-hour periods, and the identity of urinary metabolites and the rate of loss (i.e, excretion rate) were determined. Following methylbenzoic acid administration, only methylhippuric acid was detected in urine. The rate of loss was greater with the methylhippuric acid treatment than with the methylbenzoic acid treatment. This study is limited by the fact that it was conducted on a single individual. The determination that loss of xylene is limited by the availability of glycine suggests that the rate of utilization of glycine may vary with such factors as age and nutritional status of the individual.

Riihimäki and Savolainen (1980) exposed volunteers to constant levels of 100 ppm or 200 ppm or to fluctuating concentrations with peak concentrations of 200 ppm or 400 ppm xylene, either while at rest or with intermittent periods of exercise on an ergonomic bicycle. The uptake of xylene varied with ventilation and exercise. The loss of xylene from the blood followed biphasic, first-order kinetics, with the initial loss of xylene having a half-life in the venous blood of 0.5–1 hour, followed by a second phase with a half-life of 20–30 hours. The authors proposed that the two phases representing the rapid loss of xylene from the blood, mostly through conversion to methylhippuric acid followed by excretion, indicate that well-perfused organs reach equilibrium within minutes and muscles reach equilibrium within a few hours, whereas adipose tissues may require several days of continuous exposure to reach equilibrium.

In a study by Sedivec and Flek (1976a), workers who had been exposed to 200 mg/m^3 and 400 mg/m^3 xylene vapor for 8 hours were tracked at 10-minute and then 30-minute intervals for loss of xylenes in expired air and metabolites in urine and, on the second day, at 4- and 8-hour intervals for loss in expired air. The loss of xylene through the lungs gave a standard desaturation curve reflecting first-order kinetics, where the amounts of xylene recovered from the lungs decreased continuously for 24 hours following termination of exposure. Detectable amounts of unmetabolized xylene were present in expired air on the second day postexposure. The values given for the amounts of absorbed xylene lost through the lungs were 5.3, 5.8, and 3.5% for the o-, m-, and p- isomers, respectively. The authors considered the last value to reflect a higher rate of metabolism for the p-isomer relative to the other isomers. When administered as a mixture of isomers, 4.8% of the absorbed p-xylene was lost through the lungs. The loss of unmetabolized xylene in urine appeared 2 hours following the beginning of exposure but remained low throughout the study.

In the aforementioned study by Carlsson (1981), male rats were exposed to 208 mg/m^3 ^{14}C- p-xylene for 1, 2, 4, and 8 hours and the amount of xylene equivalents (xylene and its metabolites) were determined. Following rapid absorption of xylene and its broad distribution throughout the body, there was a rapid loss from the various tissues. The rate of loss of the parent material varied with concentration and period of exposure. Tissue concentrations determined 1 to 6 hours after the end of exposure were consistently lower than those recorded in

the same tissues immediately after cessation of exposure. The half-life of xylene equivalents in the subcutaneous fat was estimated to be 2.2 hours following 1-hour exposure and 6.9 hours following 8-hour exposure. The high concentration of xylene in the fat and the longer persistence of xylene during the post-exposure period most likely represents its high lipophilicity and hence its high K_{ow}.

The elimination of xylenes in urine was measured as unmetabolized xylene, toluic acid (methylbenzoic acid), and toluric acid (methylhippuric acid) (Sedivec and Flek, 1976a). Analysis of fresh urine did not indicate any toluic acid. However, following storage at ambient temperatures for "several days," toluic acid appeared in urine from the same sampling. The authors attributed this to the enzymatic hydrolysis of methylhippuric acid to the acid and the amine, mediated by microbial contamination. In other studies (Bray et al., 1949; Ogata et al., 1980), glucuronic acid conjugates have been detected in urine. The authors stated that with their analytical capabilities they were unable to directly measure the amount of glucuronic acid conjugates in the urine, but they noted that since the amount of toluic acid in the aged sample was the same as that of methylhippuric acid in the fresh urine, they presumed that no glucuronic acid or other conjugates were produced.

Although the differences between this study and others demonstrating the presence of glucuronic acid conjugates in rabbits following xylene exposure may be due to species-specific differences in metabolism, the authors stated that the dosing regimen is the decisive factor. In Bray et al. (1949), the administered dose was 0.6 g/kg; in Sedivec and Flek (1976a) the animals absorbed 0.019 g/kg xylene, or doses 30-fold lower. Overall, loss of unmetabolized xylene in urine accounted for less than 0.005% of the amount of xylene retained in the system.

In addition to monitoring the loss of unmetabolized xylene in expired air and urine and the corresponding acid or glucuronic acid, Sedivec and Flek (1976a) tracked the course of excretion of methylhippuric acid following inhalation exposure. They detected methylhippuric acid in urine following 2 hours of exposure; concentrations increased during exposure and peaked 2 hours following termination of exposure. Following the 2-hour post-exposure sampling, the amount of methylhippuric acid in urine decreased rapidly, but it was still detectable in the urine 4–5 days following exposure. When administered individually, there were no significant differences between the three isomers in the amount of methylhippuric acid excreted; however, when administered in a 1:1:1 ratio, the authors found that p-toluic acid derivatives constituted a larger proportion (41.5%) than m-toluic acid (34.7%) or o-toluic acid (23.8%) in the first 2-hour sampling, which may reflect a preferential oxidation of the p-isomer. Finally, in addition to the methylhippuric acid derivatives, the authors also recovered a small amount of xylenols, which appeared in the initial 2-hour sampling but did not increase over time. Overall, methylhippuric acids accounted for 97.1, 99.2, and 95.1% of the absorbed o-, m-, and p-isomers, respectively, and xylenols for 0.86, 1.98, and 0.05%, respectively.

Recent studies have used the level of methylhippuric acids in the urine as a quantitative indicator of xylene. Sedivec and Flek (1976b) measured the amount of toluic acids (methylhippuric acids) in urine as an indicator of exposure. Human volunteers were exposed to

200 or 400 mg/m^3 for 4 or 8 hours with a 2-hour break. The amount of excreted metabolites increased exponentially, reaching a maximum at the end of the exposure and decreasing exponentially thereafter. The authors noted that the most reliable correlation of exposure with methylhippuric acid was found by relating the concentration of the metabolite to ventilation rate, a factor that most likely has a direct relationship with the amount of xylene taken into the body.

David et al. (1979) tracked the loss of m-xylene through urine in the form of methylhippuric acid in humans exposed to 400 mg/m^3 m-xylene. The methylhippuric acid metabolite first appeared in urine within 2 hours of the start of exposure and reached a peak between 7 to 8 hours into the study (towards the end of the exposure period). Immediately following cessation of exposure the concentration of methylhippuric acid decreased significantly. Levels of methylhippuric acid were low but still detectable for 20 hours following the start of the study. The authors conducted comparable studies on Wistar-derived male rats. The treated rats received the equivalent of 50 mg/kg-day for 3 days prior to the start of the study. The rats were exposed to m-xylene concentrations ranging from 400 to 2000 mg/m^3. At exposure concentrations above 800 mg/m^3, rats pretreated with phenobarbital demonstrated an increased ability to metabolize xylene.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Physiologically based pharmacokinetic (PBPK) models for m-xylene inhalation have been developed for both rats (Tardif et al., 1991; 1992; 1993a; Kaneko et al., 2000) and humans (Tardif et al., 1993b, 1995; Haddad et al., 1999) (Appendix B). Conceptually, the models consist of five dynamic tissue compartments representing the lung, adipose tissue, slowly perfused tissues, richly perfused tissues, and the liver. Inhalation of m-xylene is represented by addition of m-xylene to the system via the lung component. It should be noted that the models lack an oral input component; thus, their use in extrapolating toxicity data across these two routes is precluded.

Concentration in arterial blood is predicted on the basis of the existing venous blood concentration, the rate of m-xylene exhalation, the inhaled m-xylene concentration, and the blood/gas partition coefficient. The concentration in each tissue compartment is predicted on the basis of the existing concentration and the arterial concentration, using appropriate tissue-blood coefficients. Metabolism is assumed, for the purposes of the models, to occur only in the liver compartment and is described by a series of equations. The venous concentration is calculated as a mean concentration, based on the blood flow rates from each compartment and the concentration of blood leaving each compartment.

Validation of the models following inhalation exposure in both rats (Tardif et al., 1993a, 1997) and humans (Tardif et al., 1995, 1997) has been reported. These models have also been applied to mixtures containing xylenes and other aromatic solvents (Haddad et al., 1999; Tardif et al., 1991, 1992, 1993a, b, 1995, 1997).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS

On December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. EPA, 2001). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the supporting studies discussed in this Toxicological Review are third-party studies; however, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. In addition, the studies cited provide data which suggests and inform a public health concern for xylenes, but were not designed or used as principal studies in the derivation of any quantitative value for xylenes based on NOAELs or LOAELs. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies that intentionally dose human subjects with toxicants to identify or quantify their effects.

4.1.1. Cancer Studies

Arp et al. (1983) conducted a reanalysis of a cohort of rubber industry workers to study the relationship between occupational exposure to benzene and other solvents and lymphocytic leukemia. Worker exposure was reconstructed using company records. The cohort from which the cases and controls were identified was defined as all active or retired hourly workers 40 to 84 years of age who were alive as of January 1, 1964, with mortality followup through December 31, 1973. Solvent exposures were inferred from groupings of occupational titles and their associated activities, which were used to estimate the potential for exposures. The authors used titles and longevity in the position to estimate periods of exposure to specific solvents, but they authors provide no indication of exposure levels. Solvent composition was inferred from records of formulations of raw materials. The time periods for which the cohorts were exposed are important because of the change from coal-based to petroleum-based sources of commercial aromatic solvents that occurred during the 1940s. The authors defined exposure as cumulative periods greater than 12 months.

The reporting of the data does not specifically address exposure to petroleum-based xylenes but does consider exposure to benzene and to secondary solvents, including xylenes.

The odds ratios (OD) for lymphocytic leukemia from exposure to solvents other than benzene, including xylenes, was 4.50 (p=0.08). However, when this value was broken down, coal-based xylenes produced an OD of 5.50 (p=0.02, 6 cases), compared with all petroleum-based solvents (principally xylenes) (OD = 1.50, p=0.41, 11 cases). No data were provided for direct exposure to xylene.

The most pronounced effect noted was the difference between exposure to coal-based solvents (OD = 6.67, p=0.01, 8 cases) and exposure to petroleum-based solvents (OD = 1.50, p=0.41, 11 cases). The source of the solvents (which, as noted above, changed during the 1940s) indicates the composition of contaminants in the mixture. Petroleum-based solvents typically have straight-chain aliphatic alkanes, whereas coal-based solvents are expected to have polycyclic aromatics as contaminants, several of which (dimethylbenzanthracene, benzo(α)pyrene and methylcholanthrene) have been shown to cause cancer in laboratory animals.

Several shortcomings of the study are noted. The small sample size (15 cases and 30 controls) limited the reliability of the observed associations, and chance could not be ruled out as a possible explanation. Some exposure misclassification was likely because exposures were inferred from solvent use histories that were of limited precision. Finally, it was noted that there may not have been a sufficient latent period for the development of tumors in workers exposed to petroleum-based solvents.

Wilcosky et al. (1984) conducted a reanalysis using the same cohort as Arp et al. (1983). This study also employed workers of the same age group of 40 to 79 for the same 10-year period, and exposures were estimated on the basis of occupational titles. Individuals were considered exposed when they had held a job title that involved direct contact with a chemical for 12 months. However, in the Wilcosky et al. study exposures were broken down into individual chemicals. The authors found a statistically significantly increased OD of 3.7 (p<0.05, 4 cases) for lymphosarcoma and a nonstatistically significant increase (OD = 3.3, 4 cases) for lymphatic leukemia resulting from exposure to xylenes. Despite the findings in Arp et al., no breakout for exposure to coal-based solvents or petroleum-based solvents was performed. Confounding issues associated with this study include the finding of a negative correlation between solvent exposure and lung cancer and the limited number of cases for each.

In brief, although the entire cohort comprised 6678 workers, the number of workers who were exposed to individual solvents was considerably less (Arp et al., 1983). The authors found an increased relative-risk estimate (5.5; p<0.02; 6 cases) for lymphocytic leukemia in workers exposed to coal-based xylenes. The reanalysis by Wilcosky et al. (1984) found an increased OD for lymphosarcomas (3.7; p<0.05) and a statistically insignificant increase for lymphatic leukemia (3.3) in workers exposed to xylenes; only 4 cases of each were detected.

Spirtas et al. (1991) conducted a retrospective cohort mortality study of 14,457 workers who worked for at least 1 year between January 1, 1952, and December 31, 1956, at an aircraft maintenance facility. The members of the cohort were followed for mortality determinations until 1982. The study was designed to evaluate health effects arising from exposure to

trichloroethylene, although exposures to all solvents were evaluated by surveying the base, interviewing long-term employees, and looking at historical files. An increased standardized mortality ratio (SMR) for cancer of the central nervous system (CNS) was observed in male workers (SMR = 1436; 95% CI = 174–5184). No increases in SMRs for multiple myeloma or non-Hodgkin's lymphoma were observed in male or female employees exposed to xylene.

This study has several limitations: the number of person-years of the workers exposed to xylene was small (1837 for males and 444 for females), the composition of xylene was not specified, concentrations to which the workers were exposed were not determined, and the confounding effect of concurrent exposure of workers to other solvents was not accounted for.

Gérin et al. (1998) conducted a population-based case-control study in Montreal, Canada. The authors identified sites of "high" incidence of cancer. The study involved the administration of questionnaires about hospitalized individuals who were being treated for cancer. The questions involved information about the lifestyles and work habits of the patients. This information was used to identify potential exposure to benzene, toluene, xylene, and styrene; exposure was semiquantitatively categorized into low, medium, or high. The researchers used randomly selected individuals to serve as controls. Although an increased OD was reported for exposure to "high" concentrations of xylene and cancer of the colon (SMR = 5.8; 8/429 for cases, compared with 3/955 for controls) and rectum (SMR = 2.7; 5/213 for cases, compared with 8/937 for controls), the number of cases was small, exposure concentrations were not defined, the xylene composition was not characterized, and approximately 88% of those exposed to xylene were also exposed to toluene and benzene. This last point is significant because the authors note that statistically significant associations were found between each of the four compounds and rectal cancer.

4.1.2. Noncancer Studies

4.1.2.1. Cohort and Case-Control Studies

Surveys conducted by Uchida et al. (1993) in factories in China identified workers exposed to solvents in the production of rubber boots or plastic coated wires or in printing work. The surveys identified 994 solvent-exposed workers. To identify and quantify solvent exposures, the workers were equipped with a diffusive air sampler for an entire 8-hour working shift. A total of 175 xylene-exposed workers (107 men, 68 women) for whom the sum of the three isomers accounted for 70% or more of the total solvent exposure (on a ppm basis) were selected for the study. The next day these workers underwent a medical examination that included subjective symptoms, clinical signs, and quantitative health measurements of hematology, serum biochemistry, and urinalysis. Controls were 241 nonexposed workers from the same factories or from factories in the same region. Both groups had worked for an average of 7 years with no change in the workplace during their working life, were of similar ages, and had comparable drinking rates and smoking habits. Subjective symptoms were evaluated by means of a survey inquiring about symptoms experienced during the work shift and another

survey of symptoms observed outside of work in the previous 3-month period. The prevalence of the subjective symptoms was calculated as

# of affirmative answers / (# of people in groups x # of questions) x 100%.

With the measurements of the three isomers combined, workers were exposed to a maximum concentration of 175 ppm xylenes, with a geometric mean of 14 ppm. m-Xylene was the most prevalent isomer, accounting for approximately 50% of the xylene exposure, followed by p-xylene (~30%) and o-xylene (~ 15%). Workers were also exposed to ethylbenzene (geometric mean of 3.4 ppm) and toluene (geometric mean of 1.2 ppm). n-Hexane was rarely detected, and benzene was never detected. There was little difference between men and women in the amount of solvent exposure.

The prevalence of subjective symptoms during the work shift and in the previous 3-month period was significantly higher ($p<0.01$) in exposed workers when compared with that of nonexposed workers for both men and women and both sexes combined. During the work shift, eye and nasal irritation, sore throat, and a floating sensation were increased among exposed worker of both sexes, and in the previous 3 months, nausea, nightmares, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up, poor appetite, reduced grasping power, reduced muscle power in extremities, and rough skin were increased in both sexes. When the exposed individuals were subdivided according to exposure intensity (1–20 ppm or >21 ppm xylenes), eye irritation, sore throat, and a floating sensation followed a concentration-related increase for symptoms reported during the work shift, whereas poor appetite was the only concentration-dependent symptom reported for the previous 3 months. No significant differences in measured hematology, clinical biochemistry, or urinalysis parameters were noted in exposed workers when compared with controls. A no-observed-adverse-effects level (NOAEL) was not identified.

Taskinen et al. (1986) studied pregnancy outcomes in female workers in eight Finnish pharmaceutical factories between 1973 and 1980. Among 1795 pregnancies there were 1179 deliveries, 142 spontaneous abortions, and 474 induced abortions. Rates of spontaneous abortions (100 x [# of spontaneous abortions / (sum of number of spontaneous abortions + number of births)]) were similar among women employed during the first trimester of their pregnancy (10.9%) and those workers who were not employed during their first trimester (10.6%). The corresponding rate of spontaneous abortion for all women in the region was 8.5% for the study period. A statistical comparison of these rates was not performed.

In a case-control study, each of 44 workers who had had a spontaneous abortion (cases) was matched by age to 3 workers who had given birth (controls). Information about chemical exposures during work was collected by questionnaire. In a univariate analysis, OD were not statistically significantly elevated for exposure to solvents (e.g., benzene, xylene, toluene, aliphatic hydrocarbons) or other chemicals (e.g., estrogens, “carcinogens”). In this analysis, OD were calculated for individual solvents. Using a logistic analysis of the collected data (which included estrogen exposure, solvent exposure by frequency of usage, and heavy lifting as

variables), a marginally significant OR was found for exposure to ≥4 solvents (OR = 3.5, 95% CI = 1.0–12.4), but the ORs for several other measures of frequency of solvent exposure (e.g., 1-3 solvents, toluene ≥once a week) were not statistically significantly elevated.

In a later case-control study (Taskinen et al., 1994), 206 women who had had spontaneous abortions and 329 age-matched referent women who had had normal births were identified among Finnish female laboratory workers during the period 1970 to 1986. Exposure information was collected by questionnaire. In a multivariate analysis that included adjustments for employment, smoking, alcohol consumption, parity, previous miscarriages, failed birth control, and febrile disease during pregnancy, statistically significant associations were found for spontaneous abortions and exposure to 3 out of 20 solvents for which questionnaire information was collected. The elevated ORs were 3.5 (95% CI = 1.1–11.2) for formalin, 4.7 (95% CI = 1.4–15.9) for toluene, and 3.1 (95% CI = 1.3–7.5) for xylene. Each of these ORs was for an exposure of 3–5 days/week; ORs were not significantly elevated for 1–2 days of exposure per week. Most of the women who reported having exposure to formalin or xylene worked in pathology or histology laboratories.

In a case-control study of 36 women who gave birth to children with congenital malformations (and 105 referent women), no statistically significant associations were found with exposure to the solvents for which questionnaire information was collected.

The Taskinen et al. (1986, 1994) studies and several others of similar design reviewed by IARC (1989) are of limited usefulness in assessing the potential reproductive toxicity of xylenes because the numbers of cases of spontaneous abortions were small and the women had been exposed to a number of chemicals.

4.1.2.2. Case Reports

Morley et al. (1970) reported the cases of three workmen exposed to approximately 10,000 ppm xylene for 19 hours. One man was dead upon arrival at the hospital. Autopsy revealed severe pulmonary congestion with focal alveolar hemorrhage and acute pulmonary edema, hepatic congestion with swelling and vacuolization of many cells in the centrilobular areas, and microscopic petechial hemorrhages in both the grey and white matter of the brain. In addition, evidence of axonal neuronal damage was indicated by swelling and loss of Nissl substance.

Another man was admitted to the hospital unconscious, exhibiting only a slight response to painful stimuli. He was also hypothermic, had a flushed face, and had peripheral cyanosis. Medium-grade moist sounds were present in his lungs, and a chest x-ray revealed patchy diffuse opacity in both lungs. Five hours following treatment with tracheal aspiration and oxygen, the patient regained consciousness but was amnesic for 2–3 days. Evidence of renal damage was indicated by an increase in blood urea of 59 mg/100 mL to 204 mg/100 mL 3 days after admission. Endogenous creatinine clearance was also reduced at this time. Slight hepatic

impairment was indicated by a rise in serum transaminase to 100 i.u. over 48 hours, followed by a return to normal levels.

The third man recovered consciousness following admission to the hospital. He was confused and amnesic, had slurred speech, and was ataxic upon walking. Within 24 hours of admission, he was fully conscious and alert, and the ataxia disappeared over 48 hours. There was no evidence of renal impairment, and mild hepatic impairment was indicated by a slight rise in serum transaminase (52 i.u.) over 48 hours, followed by a return to normal levels.

Abu-Al-Ragheb et al. (1986) reported on a 27-year-old man who committed suicide by ingesting xylene. Histopathologic findings included areas of pulmonary edema and congestion. The probable cause of death was attributed to respiratory failure and asphyxia, a secondary response elicited by depression in the respiratory center of the brain. In another case (Recchia et al., 1985), accidental ingestion of xylene resulted in a deep coma lasting more than 26 hours, hepatic impairment, hematemesis, acute pulmonary edema, and other pulmonary complications. Another individual who attempted to commit suicide by the intravenous injection of 8 ml of xylene developed acute pulmonary failure within 10 minutes of administration (Ševčik et al., 1992). The individual survived following appropriate treatment in the hospital for the respiratory effects elicited by the xylene.

Two case reports of seizures following exposure to xylene-based products have been published. Goldie (1960) reported a case where eight painters were exposed to paint that contained an 80% xylene/20% methylglycolacetate solvent. The workers complained of headache, vertigo, gastric discomfort, dryness of the throat, and slight drunkenness after 30 minutes of exposure. After 2 months of exposure, an 18-year-old worker exhibited behavior and symptoms indicative of a convulsive seizure, including weakness, dizziness, inability to speak, unconsciousness, eye and head rotation to one side, chewing but no foaming, and kicking motions. The subject recovered consciousness 20 minutes later. Arthur and Curnock (1982) reported another case in which that an adolescent worker developed major and minor seizures following the use of a xylene-based glue used for building model airplanes. Neither case report provided an exposure concentration, and exposures were not limited to xylene alone.

Klaucke et al. (1982) reported that 15 workers who had been exposed by inhalation to xylenes were admitted to a small community hospital, each complaining of at least two of the following symptoms: headache, nausea, vomiting, dizziness or vertigo, eye irritation, and nose or throat irritation. The frequency of the symptoms were headache: 12/15; nausea: 10/15, eye irritation: 8/15; nose or throat irritation: 7/15; dizziness or vertigo: 7/15; and vomiting: 6/15. Fourteen of the 15 affected employees noted an unusual odor 15–30 minutes prior to the onset of symptoms. It was estimated that the workers were exposed to levels as high as 700 ppm.

Five women occupationally exposed to inhaled xylene from 1.5 to 18 years experienced symptoms that included chronic headache, chest pain, electrocardiogram abnormalities, dyspnea, cyanosis of the hands, fever, leukopenia, malaise, impaired lung function, decreased ability to work, complete disability, and mental confusion (Hipolito, 1980).

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Prechronic Studies

4.2.1.1. Prechronic Oral Studies

Groups of 10 male and 10 female Fischer 344 rats were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene, and 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 62.5, 125, 250, 500, or 1000 mg/kg-day for 5 days per week for 13 weeks (NTP, 1986). At termination of the study, necropsy was performed on all animals and comprehensive histologic examinations were performed on vehicle and high dose-group animals. High-dose males and females gained 15% and 8% less body weight, respectively, than did controls, with final body weights being 89% and 97%, respectively, of those of controls (statistical significance not reported). No signs of toxicity or treatment-related gross or microscopic pathologic lesions were observed. The lowest-observed-adverse-effect level (LOAEL) is 1000 mg/kg-day, based on decreased body weights in male rats, and the NOAEL is 500 mg/kg-day.

In the same study, male and female B6C3F_1 mice were treated with mixed xylenes. Groups of 10 mice of each sex were administered 0, 125, 250, 500, 1000, and 2000 mg/kg-day in corn oil by gavage 5 days per week for 13 weeks. Two female mice in the high-dose group died prematurely, although gavage error could not be ruled out as the cause. At 2000 mg/kg-day, the animals exhibited lethargy, short and shallow breathing, unsteadiness, tremors, and paresis starting at 5–10 minutes after dosing and lasting for 15–60 minutes. Mean body weight of the mice in the high-dose group was 7% lower than in the vehicle control for males and 17% lower for females. Although it was not stated explicitly, the report implies that this was a common finding among the animals dosed at this level. No treatment-related gross or microscopic pathologic lesions were seen in this study. The NOAEL is 1000 mg/kg-day and the LOAEL is 2000 mg/kg-day for transient signs of nervous system depression.

In a study by Condie et al. (1988), groups of 10 male and 10 female Sprague-Dawley rats were administered mixed xylenes (17.6% o-xylene, 62.3% m-xylene and p-xylene (which coeluted); 20% ethylbenzene) by gavage in corn oil for 90 consecutive days at doses of 0, 150, 750, or 1500 mg/kg-day. Effects of exposure included decreased body weights in high-dose males (94% of controls'); dose-related increased liver weights and liver-to-body weight ratios in all exposed groups of males (8, 18, and 29% increase in absolute weight above controls in the low-, mid-, and high-dose animals, respectively) and in mid- and high-dose females (14 and 30% increases in absolute weight above controls); and increased kidney weights and kidney-to-body weight ratios in mid- and high-dose males (16 and 19% increase in absolute weight relative to controls, respectively) and high-dose females (18% increase in absolute weight relative to controls). The authors postulated that the modest increases in aspartate aminotransferase (AST) seen in high-dose females and increases in alanine aminotransferase (ALT) in high-dose males and in mid- and high-dose females, combined with the lack of significant histopathologic

findings in the liver suggest that the enlargement of the liver was an adaptation response to xylene treatment rather than an adverse toxicological effect.

Hematology analysis revealed a mild polycythemia and leukocytosis in the high-dose males and females in the absence of any observable changes in the health of the rats. Microscopic evaluation of the kidneys revealed a dose-related increase in hyaline droplet formation in male rats (0/9, 3/9, 5/10, 8/10, respectively) and a dose-related increase in the early appearance of minimal chronic nephropathy only in female rats (1/10, 3/10, 6/10, 7/10, respectively). Compared with controls, the incidence of minimal nephropathy was statistically significantly elevated ($p<0.05$) in the 750 and 1500 mg/kg-day female groups but not in the 150 mg/kg-day group (Fisher Exact test performed by Syracuse Research Corporation). The hyaline droplet formation in male rats was assumed by the authors to be related to male rat-specific α-2μ-globulin accumulation and not to be relevant to humans. The LOAEL is 750 mg/kg-day, based on increased kidney weights and early appearance of nephropathy in female rats, and the NOAEL is 150 mg/kg-day.

In a study by Bowers et al. (1982), aging (12–19-month-old) Long-Evans hooded, male rats were administered methylated benzenes, including o-xylene, in feed at a concentration of 200 mg/kg feed (10 mg/kg-day) for 1, 2, 3, or 6 months, to access ultrastructural changes in the liver. No other endpoints were evaluated. Although the liver was grossly normal, electron microscopic evaluation revealed two types of membrane-bound vacuoles in hepatocytes appearing 1 month after beginning administration of the feed. The appearance and size of the vacuoles did not change with continued dietary administration of the compound.

In a study by Wolfe (1988a), groups of 20 male and 20 female Sprague-Dawley rats were administered m-xylene (99% purity) by gavage in corn oil at doses of 0, 100, 200, or 800 mg/kg-day for 90 consecutive days. Survival incidences were 20/20, 17/20, 15/20, and 18/20, respectively, for males and 20/20, 20/20, 16/20, and 16/20, respectively, for females. Mortality in the mid-dose males and mid- and high-dose females attained statistical significance ($p\leq0.05$), but a significant trend was observed only in females. Mottled lungs and a failure of the lungs to collapse were observed in all mid- and high-dose animals that died early and in 2/3 of the low-dose males that died early but were not evident in any of the animals that survived to study termination. Histopathologic examination of the lungs from animals that died before study termination revealed foreign material in the alveoli in all but one animal. Therefore, these deaths were attributed to vehicle and/or compound aspiration.

Clinical signs present throughout the study were limited to high levels of salivation prior to dosing in high-dose males and females. Body weight gains over the entire study period were decreased ($p\leq0.05$) in mid- and high-dose males (89% and 75%, respectively, of controls') and high-dose females (85% of controls'). Food consumption was likewise decreased ($p\leq0.05$) in high-dose males during weeks 1–5 (90% of control levels) and in mid- and high-dose males during weeks 6–9 (92% of control levels for both groups). A thorough histologic examination revealed no other abnormalities. Other effects noted were not definitively related to treatment

and/or were not biologically significant. The NOAEL and LOAEL are identified as 200 and 800 mg/kg-day, respectively, based on decreased body weight.

In a second study by Wolfe (1988b), groups of 20 male and 20 female Sprague-Dawley rats were administered p-xylene (99% purity) by gavage in corn oil at doses of 0, 100, 200, or 800 mg/kg-day for 90 consecutive days. Survival incidences were 20/20, 19/20, 17/20, and 16/20, respectively, for males and 20/20, 18/20, 18/20, and 17/20, respectively, for females. Mortality in high-dose males attained statistical significance, and a statistically significant trend was present in the male groups. As in the Wolfe (1988a) study, mottled lungs and/or a failure of the lungs to collapse was observed in nearly all treated animals that died early but was not evident in any of the animals that survived to study termination. It was determined that most of the unscheduled deaths were the result of test material aspiration, as indicated by the presence of intra-alveolar foreign material in the lungs that was generally associated with pulmonary congestion.

Treatment-related clinical signs were limited to increased salivation occurring just prior to dosing that was resolved by 1-hour post dosing in both high-dose males and females. Body weight gains at 13 weeks were slightly reduced in high-dose males and females (89% of control levels, not statistically significant), and high-dose females had significantly increased food consumption for weeks 10–13 (110%). No treatment-related effects were observed in hematology or clinical chemistry parameters, ophthalmologic examination, or organ weights. Histopathology revealed no abnormal findings in any tissue or organ. The NOAEL and LOAEL are identified as 200 and 800 mg/kg-day, respectively, based on early mortality in male rats that showed signs of test material aspiration into the lungs.

In a nephrotoxicity screening study by Borriston Laboratories, Inc. (1983), groups of 10 male Fischer 344 rats were dosed with 0.5 or 2.0 g/kg m-xylene or 2.0 g/kg saline by gavage for 5 days/week for 4 weeks. No nephrotoxic effects were observed in the rats dosed with m-xylene when compared with controls.

4.2.1.2. Prechronic Inhalation Studies

In a study by Carpenter et al. (1975), groups of 25 male rats and 4 male beagle dogs were exposed to air containing measured concentrations of 180, 460, or 810 ppm mixed xylenes (65.0% m-xylene, 7.8 % p-xylene, 7.6% o-xylene, 19.3% ethylbenzene) for 6 hours per day, 5 days per week, for 65 days (rats) or 66 days (dogs). Endpoints assessed included changes in body weight, hematology, clinical chemistry, urinalysis, organ weights, and histologic examination. Three rats from each dose level were sacrificed for histologic evaluation after 15 and 35 days of exposure. Additional measurements in dogs included food consumption and initial and terminal electrocardiograms.

No treatment-related effects in any of the measured parameters were observed in exposed rats or dogs when compared with control animals. Additionally, 10 rats per dose, including a control group handled similarly to the exposed rats and a group of 10 naive control rats, were

challenged with a 4-hour exposure to 6700 ppm xylene (29.0 mg/L) at the termination of the subchronic exposure period. No difference in the median time to death was noted in rats exposed to xylenes for 65 days when compared with control rats. Therefore, the highest exposure level in this study (810 ppm) is a NOAEL for changes in body weight, organ weights, and histopathology in male rats and male beagle dogs.

Tátrai and Ungváry (1980) exposed groups of 30 male CFY rats to 0 or 3500 ppm o-xylene (purity not stated) for 8 hours per day for 6 weeks. Following exposure, body weights and organ weights were measured. Organs of 5 rats in each group were examined by light microscopy and livers were also examined by electron microscopy. Group means and standard errors were calculated with Student's one- and two-sample t-test. Despite increased food and water consumption, terminal body weight at 6 weeks in the xylene-exposed group was statistically significantly lower ($p<0.05$) than in controls (427.50 ± 7.08 g vs. 454.00 ± 10.40 g). Exposed rats had hepatic changes, including increased absolute and relative liver weights, signs of hepatocellular hypertrophy, increased proportion of smooth and rough endoplasmic reticulum, decreased glycogen, and increased peroxisomes. Measurements of drug metabolizing enzymes were not made. The observed changes in organ weight were consistent with an adaptive response to organic chemical exposure and probably reflected induction of enzymes in the liver. An explanation for the differences in body weight was not provided. The only exposure level in this study, 3500 ppm o-xylene, is a LOAEL for statistically significant body weight decreases of about 6% in male rats showing adaptive, but not adverse, changes in the liver.

To investigate the potential for exposure to xylene to induce hepatotoxic effects, Tátrai et al. (1981) exposed male CFY rats to air containing 0 or 1090 ppm (4750 mg/m^3) o-xylene for 8 hours per day, 7 days per week, for 6 or 12 months. The purity of the o-xylene was not provided. Biochemical indices of liver metabolic capacity (drug-metabolizing enzyme activities) and liver damage (serum activities of AST and ALT; reported as "GOT" and "GPT") were measured, as were body weights and organ weights. Liver tissue samples were examined by light and electron microscopy. Statistical differences between exposed and control group means were compared by variance analysis and Dunnett's test.

Exposure to 1090 ppm o-xylene for 6 or 12 months resulted in increased food and water consumption, decreased body weight gain, increased absolute and relative liver weight, and induction of enzymes of the hepatic mixed-function oxidase system (increased cytochromes P-450 and b-5, cytochrome c reductase, alanine hydroxylase, and aminopyrene N-demethylase). Data were presented in graphical form only. At 1 year, the control mean body weight was about 700 g, compared with an exposed mean of about 600 g. This change represents about a 15% decrease and was reported to have been statistically significant ($p<0.05$).

Histologic and histochemical examination of the organs, including the liver, were reported to have shown no pathological alterations or changes in serum activities of AST or ALT. Electron microscopy of liver tissue samples revealed moderate proliferation of the smooth endoplasmic reticulum. The hepatic effects are not considered adverse. The exposure level of

1090 ppm o-xylene is a LOAEL for decreased body weight in male rats with no adverse changes in hepatic endpoints.

Ungváry (1990) exposed groups of male CFY rats to air containing 0, 140, 350, or 920 ppm (0,600, 1500, or 4000 mg/m^3) xylenes (10% o-xylene, 50% m-xylene, 20% p-xylene, 20% ethylbenzene) for 8 hours per day, 7 days per week for 6 weeks and then for 5 days per week for 6 months. Endpoints evaluated and statistical evaluations were the same as those in earlier reported studies from this investigator's laboratory (Tátrai and Ungváry, 1980; Tátrai et al., 1981). No statistically significant differences in body weights were observed in any of the exposed groups when compared with the control values.

Statistically significant changes observed in exposed groups at 6 months (compared with control group values) included increased relative liver weight (17% in the high-dose group only); hypertrophy of the centrilobular zone of the liver (high-dose group only); increased nuclear volume of hepatocytes and proliferation of smooth endoplasmic reticulum (only the high-dose and control groups were examined); increases in the concentrations of cytochrome P-450 and cytochrome b_5 (mid- and high-dose groups); increases in the activities of NADPH, cytochrome c-reductase, alanine p-hydroxylase, succinate dehydrogenase and aminopyrine N-demethylase (mid- and high-dose groups); and decreased hexobarbital sleeping time (mid- and high-dose groups). In general, maximal effects were achieved by 6 weeks of exposure; control levels returned after a 4-week solvent-free period following the 6-month exposure.

In further experiments reported by Ungváry (1990), continuous inhalation exposure of CFY rats to 0, 350, 460, or 1150 ppm (0,1500, 2000, or 5000 mg/m^3) for 72 hours or repeated inhalation exposure of male mice, rats, or rabbits to 0 or 575 ppm (2500 mg/m^3) for 8 hours per day for 6 weeks resulted in effects similar to those reported for the repeated exposure study in male rats for 6 months. Lastly, continuous exposure to 0 or 690 ppm xylenes (3000 mg/m^3) for 72 hours in male CFY rats following partial hepatectomy or bile duct ligation still induced the biotransformation enzymes but did not appear to potentiate the damage induced by these two interventions. The authors did not consider the observed effects to be adverse and identified the highest exposure level (920 ppm) to be a NOAEL. However, the liver effects could be considered biologically relevant, indicating a LOAEL of 920 ppm and a NOAEL of 350 ppm.

Jenkins et al. (1970) conducted inhalation studies on 12 Sprague-Dawley or Long Evans rats, 15 NMRI:(ASH) Princeton-derived guinea pigs, 2 squirrel monkeys, and 2 beagle dogs in which the animals were repeatedly exposed to 780 ppm o-xylene for 8 hours per day, 5 days per week, for a total of 30 exposures over a 6-week period. In another portion of the study, 14 rats, 15 guinea pigs, 2 dogs, and 3 monkeys were exposed to 78 ppm o-xylene continuously for 90–127 days; 14 rats, 15 guinea pigs, 10 dogs, and 12 monkeys were exposed continuously for 90–127 days to control air to serve as reference groups. During the 780 ppm study, two rats died on the third day of exposure, one rat and one monkey died on the seventh day of exposure, and one of the dogs exhibited tremors throughout the exposure. The cause of death and any clinical signs occurring before death were not reported. No changes in body weights, hematology

parameters, or histopathology in animals exposed to 78 or 780 ppm were reported. A LOAEL/NOAEL is not determined because of inadequate data reporting.

In a study by Morvai et al. (1976), 16 CFY rats exposed to xylene (composition not stated) developed respiratory paralysis preceded by atrial fibrillation, bradyarrhythmia, and asystole. The conditions of the exposure were not clear; exposure was assumed to be 1400 ppm (6000 mg/m^3) for 6 hours per day.

4.2.2. Chronic Studies and Cancer Assays

4.2.2.1. Oral Studies

In National Toxicology Program toxicology and carcinogenesis studies (NTP, 1986), groups of 50 male and 50 female Fischer 344 rats and 50 male and 50 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene, 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 250, or 500 mg/kg-day (rats) and 0, 500, or 1000 mg/kg-day (mice) for 5 days per week for 103 weeks. Necropsy and histologic examinations were performed on all animals. Tissues were examined for gross lesions and masses. The tissues examined included mandibular lymph nodes, salivary gland, femur (including marrow), thyroid gland, parathyroids, small intestine, colon, liver, prostate/testis or ovaries/uterus, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, skin, lungs and mainstem bronchi, kidneys, adrenal glands, urinary bladder, pituitary gland, eyes (if grossly abnormal), and mammary gland. Hematology and clinical chemistry analyses were not conducted.

In rats, no statistically significantly increased incidences of nonneoplastic or neoplastic lesions (that would have been expected to be treatment-related) were found in exposed groups when compared with controls. The authors noted that a survival-adjusted increased incidence of interstitial cell tumors was found in the high-dose male rat group relative to controls, but they did not consider this to be a treatment-related increase. The increase was attributed to high-dose male rats dying between weeks 62 and 92 and it was noted that incidences for these tumors were comparable with those of controls during other time intervals and that the overall incidences were not statistically significantly different between control and exposed groups.

Effects of exposure in rats were limited to decreased body weight and decreased survival in high-dose (500 mg/kg-day) males. Mean body weights were 5–8% lower in high-dose male rats than in controls from week 59 to week 97, with body weights at 103 weeks being 4% less in high-dose males than in controls (statistical significance not reported). Male rat survival rates after 103 weeks showed a dose-related decrease (36/50, 25/50, and 20/50 for control, low-, and high-dose males, respectively). A life-table trend test for decreased survival incidence with increasing dose was statistically significant (p=0.033). Pair-wise comparisons with control survival incidence indicated that only the high-dose male rat incidence was significantly decreased (p=0.04). A number of the deaths were attributed to gavage error (3/50, 8/50, and 11/50, respectively). The authors did not record observations of rat behavior during dosing.

Based on the available observations, the incidence of treatment-related deaths demonstrated a dose-related increase (11/50 [22%], 17/50 [34%], and 19/50 [38%]). Based on increased mortality and decreased body weight in male rats, this study identifies 500 mg/kg-day as a LOAEL and 250 mg/kg-day as a NOAEL. There was no evidence of carcinogenicity in male or female rats exposed to doses up to 500 mg/kg-day.

In mice, no statistically significantly increased incidences of nonneoplastic or neoplastic lesions were found in male or female exposed groups when compared with controls. The only treatment-related effect observed was hyperactivity, which occurred in all high-dose mice of each sex 5–30 minutes after dosing. This effect was observed consistently beginning at week 4 and continued until study termination at 103 weeks. The LOAEL is 1000 mg/kg-day and the NOAEL is 500 mg/kg-day for hyperactivity. There was no evidence of carcinogenicity in male or female mice exposed to doses up to 1000 mg/kg-day.

Maltoni et al. (1983, 1985) exposed groups of 40 male and 40 female Sprague-Dawley rats to 500 mg/kg mixed xylenes (unspecified proportions) in olive oil orally by gavage 4–5 days per week for 104 weeks. The control groups of 50 males and 50 females were treated with olive oil only. The animals were kept under observation until spontaneous death; all rats died by 141 weeks. The proportion of mice that survived treatment was similar in controls and treated groups through 92 weeks (Maltoni et al., 1983), but survival data for later periods were not reported (Maltoni et al., 1985). For example, 50% and 65% of exposed males and females, respectively, survived at 92 weeks, compared with 58% and 66% of control males and females.

Average body weights (standard errors not reported) at several intervals through 92 weeks appeared similar or higher in exposed versus control groups, but statistical significance was not evaluated. For example, exposed male rats had average body weights of 611.0 g and 626.25 g at 78 and 92 weeks, respectively, compared with control values of 525.92 g and 490.68 g. Exposed females at 78 and 92 weeks had average body weights of 413.48 g and 437.11 g, compared with control values of 356.5 g and 389.09 g. Counts of red blood cells and white blood cells from 4–5 rats per group were measured from blood collected at 84 weeks; they did not appear to be affected by exposure (Maltoni et al., 1983). Mean counts (± standard deviation) for red blood cells were 9.05 ± 0.13 for males and 7.58 ± 0.61 for females in the exposed group, compared with respective means of 8.72 ± 0.57 and 7.70 ± 0.50 for controls. Respective total white blood cell counts were 10.65 ± 0.66 and 9.00 ± 1.82 in exposed rats, compared with 2.92 ± 2.29 and 10.80 ± 2.74 in controls.

Only limited information regarding tumor incidences at specific tissue sites was provided with no information provided on nonneoplastic lesions or tumor pathology. Final (i.e., 141-week) tumor incidence data were reported only for rats with hemolymphoreticular neoplasias (thymomas, others, and total) and for rats with malignant tumors at any site (Maltoni et al., 1985). Incidences for thymomas were 1/34 and 0/36 in exposed males and females, compared with 0/45 and 0/49 in controls. Incidences of rats with other hemolymphoreticular neoplasias (not otherwise specified) were 4/34 and 3/36 in exposed males and females, compared with 3/45

and 1/45 in controls.[1] Fisher exact tests (performed by Syracuse Research Corporation) indicated no significant differences between groups in the incidences for hemolymphoreticular neoplasias (including the combined incidence for thymomas and "others").

The study authors also reported an increase in the total number of exposed rats with malignant tumors (of unspecified type): 14/38 and 22/40 for exposed males and females, compared with 11/45 and 10/49 for controls.[2] The exposed female total malignant tumor incidence was statistically significantly increased when compared with controls by the Fisher Exact test. Because of the incomplete reporting of site-specific tumor incidence data and pathology, the study by Maltoni et al. (1983, 1985) is of limited use in evaluating the carcinogenicity of xylenes. For noncancer effects, the study identifies an apparent NOAEL of 500 mg/kg for changes in body weight and counts of red and white blood cells in male and female rats.

4.2.2.2. Inhalation Studies

No chronic toxicity or cancer studies in animals exposed by inhalation to xylenes are available.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Reproductive Studies

4.3.1.1. Oral Reproductive Studies

No studies of the reproductive toxicity of xylenes following oral exposure are available.

4.3.1.2. Inhalation Reproductive Studies

In a one-generation reproductive toxicity study (Bio/dynamics Inc., 1983), groups of male and female CD rats were exposed to 0, 60, 250, or 500 ppm mixed xylenes (groups I, II, III, and IV, respectively; technical-grade xylene: 2.4% toluene, 12.8% ethylbenzene, 20.3% p-xylene, 44.2% m-xylene, 20.4% o-xylene) by inhalation for 6 hours per day, 5 days per week, for 131 days prior to mating, with exposure continued in females on gestation days (GDs) 1–20 and lactation days 5–20. Two additional 500-ppm groups were similarly exposed, except that only the F_0 males were exposed in group V, and only the F_0 females were exposed in group VI.

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[1] Denominators are the number of rats reported to have been alive at 58 weeks when the first hemolymphoreticular neoplasia was observed.

[2] The denominators are the reported numbers of rats alive at 33 weeks when the first malignant tumor was observed.

In-life parameters evaluated in adults included pre-mating body weights, observations for mortality and clinical signs, detailed weekly physical examination, maternal body weights, and maternal food consumption and food efficiency. One-half of all F_0 males were sacrificed after the mating period for gross postmortem examination; the remaining half were sacrificed and examined 21 days later. One-half of the group I F_0 females and group IV F_0 females were sacrificed on GD 21 for developmental toxicity evaluation; the results of this evaluation are described in Section 4.3.2.2. The remaining F_0 females were allowed to deliver litters.

Litters were standardized by pooling all pups within each treatment group on lactation day 4 and redistributing four males and four females from this pool to each dam. However, on some days the pups could not be pooled if only one litter was available. In this case, litters were culled to four males and four females when possible. Pups were weighed, sexed, and given a gross external examination on lactation days 1, 4, and 21. Randomly selected pups from each group (one/sex/litter) and all remaining F_0 females with litters were sacrificed on day 21 of lactation and subjected to gross necropsy. The remaining pups were maintained for the post-weaning interval of 28–49 days and weighed and sacrificed on day 49. Randomly selected pups from each group (one/sex/litter) were given a complete gross postmortem examination.

No adverse effects were noted in F_0 adults. No differences were observed in testes weights or histologic examination of reproductive tissues in xylene-exposed males sacrificed after mating when compared with control males. Although the female mating index in group III and group VI was significantly lower than for controls (85 and 85%, respectively, vs. 100% for controls), the decreases were not considered by the authors to be chemically related because a similar effect was not observed in group IV (500 ppm-exposed males and females) and also because the decreases were compared to an unusually high mating performance in the controls. The male mating index, pregnancy rate, and fertility index in exposed animals were comparable to control values. Thus, the highest exposure level in this study, 500 ppm, for 6 hours per day, 5 days per week, for 131 days before mating and continuing through weaning at day 20, is a NOAEL for reproductive endpoints evaluated in the parental generation. Developmental endpoints evaluated in this study are discussed in Section 4.3.2.2.

In a study of the possible influence of xylene or toluene co-exposure with n-hexane on testicular endpoints and reproductive function in male Sprague-Dawley rats (Nylén et al., 1989), groups of rats were also exposed to 0 or 1000 ppm xylene alone for 18 hours per day, 7 days per week, for 61 days. The xylene tested in this study was referred to as a fluid solvent obtained from GR Merck, but the composition of the material was not specified. In another study by this group of investigators (Nylén and Hagman, 1994), the test material was xylene solvent from GR Merck and was reported to contain 1.5% o-xylene, 65% m-xylene, 32% p-xylene, and 2.5% ethylbenzene.

The means and ranges for a number of male reproductive tissue variables were essentially the same for exposed and control rats (six rats per group) evaluated at 2 weeks or 10 months following exposure. Endpoints evaluated were percentages of intact spermatozoa, percentages of spermatozoa with normal heads and tails, testis weight, ventral prostate weight, and

noradrenaline concentration in vas deferens. In addition, three rats exposed to xylene alone were reported to have been fertile when tested 14 months after cessation of exposure. This study identifies 1000 ppm xylene (presumably mixed xylenes) as a NOAEL for testicular effects and fertility in male rats.

4.3.2. Developmental Studies

4.3.2.1. Oral Developmental Studies

Pregnant CD-1 mice were administered mixed xylenes (60.2% m-xylene, 9.1% o-xylene, 13.6% p-xylene, 17% ethylbenzene) by gavage in cottonseed oil three times daily at doses of 0, 520, 1030, 2060, 2580, 3100, or 4130 mg/kg-day during GDs 6–15 (Marks et al., 1982)[3]. Mice were sacrificed on GD 18 and maternal and fetal endpoints were assessed. The litter was the experimental unit for statistical analysis of the data. A p value of <0.05 was selected as the level of significance. Differences between groups were evaluated with the Mann-Whitney U-test or Fisher Exact test. Dose-response relationships were measured by Jonckheere's trend test.

The highest dose (4130 mg/kg-day) was lethal to 15/15 dams, and the 3100 mg/kg-day dose, resulted in the death of 12/38 dams and decreased maternal body weight gain (49% of controls' for GDs 1–18). Maternal body weight gains were not significantly affected at doses $\leq$2580 mg/kg-day. Average gravid uterine weight was statistically significantly decreased at doses $\geq$2060 mg/kg-day, compared with control values. Average gravid uterine weight also showed a statistically significant trend for decreasing effects with increasing dose.

The average fetal weight per litter (stunted and dead fetuses were not included) was significantly decreased in the groups treated with doses $\geq$2060 mg/kg-day. Expressed as percentages of the control value, the average fetal weights were 100, 93, 88, 80, and 72% for the 520, 1030, 2060, 2580, and 3100 mg/kg-day groups, respectively. The trend for decreasing fetal weight with increasing dose was statistically significant. The percent resorptions of total number of implants was significantly different from that of controls in only the 3100 mg/kg-day group (62.3% vs. 11.2%). Average percentages of fetuses with malformations[4], consisting primarily of cleft palate, were statistically significantly increased in groups treated with doses $\geq$2060 mg/kg-day. This variable showed a statistically significant trend for increasing malformations with increasing dose. For the control through 3100 mg/kg-day groups, the percentages were 0.3, 1.0, 1.0, 3.4, 7.8, and 9.1%, respectively.

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[3] Marks et al. (1982) noted that xylene dissolved in cottonseed oil at concentrations (v/v) of 0 (vehicle control), 2, 3, 8, 10, 12, and 16% were administered by gavage in individual doses three times a day and that the daily doses were 0, 0.6, 1.2, 2.4, 3.0, 3.6, and 4.8 mL/kg-day. It was noted that a density value of 0.86 g/mL was used to convert these to units of mg/kg-day, but the actual numbers in the report should be in units of g/kg-day. This was an apparent typographical error. The mg/kg-day doses cited herein appear to be the correct administered doses.

[4] 100 x $\sum$ (# of malformed fetuses in litter/total # of litters) / total # of fetuses in litter

Using decreased gravid uterine weight as a measure of maternal toxicity, the maternal LOAEL is 2060 mg/kg-day and the NOAEL is 1030 mg/kg-day. Maternal mortality and decreased weight gain occurred at doses ≥3100 mg/kg-day. The developmental LOAEL is 2060 mg/kg-day, based on decreased fetal body weight and increased percentage of fetuses with malformations (primarily cleft palate), and the NOAEL is 1030 mg/kg-day.

In another study reported only as an abstract (Nawrot and Staples,1980), xylene isomers (m-, o-, or p-) were administered by gavage to pregnant CD-1 mice at apparent doses of 0, 0.3, 0.75, or 1.00 mL/kg[5] three times daily on GDs 6–15 or 1.0 mL/kg three times daily on GDs 12–15. Assuming a density of 860 mg/mL, these doses correspond to 0, 774, 1935, and 2580 mg/kg-day. The abstract mentioned a control group but did not specify the number of dams in the groups. Exposure to the mid and high dose of o- or p-xylene and the high dose of m-xylene during GDs 6–15 resulted in overt maternal toxicity (not otherwise specified) and a significantly increased incidence of resorptions. The mid- and high-dose o- or p- xylene-exposed groups (GDs 6–15) additionally had an increased incidence of cleft palate (the actual incidence was not cited). Exposure to 2580 mg/kg-day of any isomer during GDs 12–15 resulted in a significant increase in maternal lethality, with exposure to p- or m-xylene additionally resulting in a significant increase in the incidence of malformations, consisting mostly of cleft palate.

Subsequent studies on the developmental effects of m-xylene were conducted: mice were administered m-xylene at doses of 1935 or 2580 mg/kg-day during GDs 12–15 or 6–15. Although exposure to m-xylene during GDs 12–15 did not result in overt toxicity at the low dose and did not significantly increase the incidence of malformations, exposure during GDs 6–15 did result in a low (4.4% vs. 0.0% in vehicle controls) but statistically significant increase in cleft palate in only the high-dose group, in the absence of overt maternal toxicity. The abstract report does not identify reliable NOAELs and LOAELs for maternal toxicity and developmental toxicity because of incomplete reporting.

4.3.2.2. Inhalation Developmental Studies

Litton Bionetics (1978a) exposed groups of pregnant CRL:COBS CD (SD) BR rats to 0, 100, or 400 ppm xylene (52% m-xylene, 11% o-xylene, 0.31% p-xylene, 36% ethylbenzene) for 6 hours per day on GDs 6–15. On GD 20, the dams were sacrificed and each uterus was removed and examined. The number of implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were removed, examined externally for abnormalities, and weighed. One third of fetuses in each litter were fixed in Bouin's fluid and later examined for changes in soft tissues of the head and thoracic and visceral organs. The remaining fetuses of each litter were examined for skeletal abnormalities following staining with Alizarin Red S. Mean body weight and food consumption were not statistically significantly affected in the

[5] The abstract (Nawrot and Staples, 1980) noted that doses were administered three times daily ("t.i.d.") from day 6-15 of gestation at 0.30, 0.75, and 1.00 mg/kg/dose and from days 12-15 of gestation at 1 mg/kg/dose. The IARC (1989) Working Group noted that the doses were incorrectly expressed as mg, rather than mL, in the abstract.

exposed dams when compared with controls. When compared with control values, no statistically significant exposure-related changes, were found in the number of live litters, number of implantation sites, number or percentage of litters with resorptions, litters with dead fetuses, mean liver litter size, or the average fetal body weight.

No exposure-related malformations were found in the fetuses, but some skeletal changes indicative of retarded bone ossification were reported in the 400 ppm group. The incidence of fetuses with retarded bone ossification was statistically significantly elevated relative to controls in the 400 ppm group but not in the 100-ppm group. The reported incidences for total fetuses examined with retarded bone ossification were 19/196 (9.7%), 24/197 (12.2%), and 37/201 (18.4%) for the control, 100, and 400 ppm groups, respectively. However, a Wilcoxon Rank Sum test (based on the number of abnormal fetuses within a litter) indicated no significant difference between the 400 ppm group and the control group. The majority of affected fetuses (27/37) were in three litters in which all of the fetuses were small. The authors interpreted the difference in retarded bone ossification between the 400 ppm and control groups to be not related to treatment. The highest exposure level in this study, 400 ppm, is judged to be a NOAEL for maternal and developmental toxicity.

In the one-generation reproductive study of CD rats described in Section 4.3.1.2 (Bio/dynamics Inc., 1983), one-half of the group I F_0 pregnant dams (20 females; control group) and group IV F_0 pregnant dams (12 females exposed to 500 ppm mixed xylenes by inhalation for 6 hours per day, 5 days per week, during a premating period and during gestation) were sacrificed on GD 21 for developmental toxicity evaluation. Gross necropsy was conducted on each animal. Maternal exposure to 500 ppm mixed xylenes did not adversely affect maternal body weights, food consumption, food utilization, or the results of postmortem examination. Corrected terminal body weights (corrected for gravid uterine weights) for exposed females were statistically significantly increased when compared with those of controls, but the increases were not considered to be biologically significant (106% of controls). Although absolute kidney weights were statistically increased in group IV females (110% of controls'), kidney weights relative to body weights were comparable to those of controls. The increase in absolute kidney weights in the exposed females was, therefore, attributed to the higher body weights.

No statistically significant differences were noted between treated (group IV) and control groups for mean number of corpora lutea, implantations, live fetuses, mean percentage of live fetuses/implants, or fetal sex ratios. Although the exposed group had an increased mean number of resorption sites (1.6 vs. 1.2 for controls) and mean percentage of resorptions to implants (16.2% vs. 9.9% for controls), the increases were not statistically significant. No dams had whole litter resorption. No definitive treatment-related external, visceral, or skeletal malformations or variations were observed. The exposed group had a slightly higher incidence of unossified sternebrae and incompletely ossified cervical vertebral transverse processes, but the incidences were provided in terms of fetal incidence instead of litter incidence. Mean fetal body weights on GD 21 were marginally but statistically significantly decreased in exposed female fetuses (93% of controls'); however, male fetal weights were comparable to those of controls. The decrease is judged to not be biologically significant.

The assessment of developmental effects in the fetuses at GD 21 in this phase of the Bio/dynamics Inc. (1983) study identified 500 ppm as a NOAEL for maternal and developmental toxicity (including assessment of external, visceral, and skeletal fetal malformations or variations).

Dams in the other exposed groups (II, 60 ppm; III, 250 ppm; V, only males exposed to 500 ppm; VI, only females exposed to 500 ppm) and the other half of the dams in group IV (males and females exposed premating F_0, 500 ppm) and the control group I were allowed to deliver their litters. No gross malformations were reported among the pups. Pups were weighed on lactation days 1, 4, 14, 21, and 49. No statistically significant decrease in mean pup body weights were observed in the exposed versus control groups at days 1 and 14. On lactation day 4, mean pup weights were statistically significantly decreased in groups II (60 ppm), III (250 ppm), and IV (500 ppm) (post-pooling) when compared with controls, but the decreases (about 8%) were not of a biologically significant magnitude. The decreased weights may have been the consequence of an elevated mean pup weight in the control group potentially caused by a smaller mean litter size (mean number of live pups per litter: 9.6, 11.8, 12.5, 12.4, 10.8, and 11.8 for groups I–VI, respectively).

Pups from group IV had statistically significant decreased mean pup weights on lactation day 21 (90% of controls') and statistically significant decreased terminal body weights at 49 days of age (as a percentage of controls': males, 92%; females, 93%). However, despite the marginal decreases observed in mean pup weights in group IV, no decreases in body weights were observed in pups from group VI, in which dams were exposed to the same concentration of xylene (500 ppm) for the same period of time as were dams in group IV. Due to the inconsistency between the fetal body weights in groups VI and IV and the small mean litter size in the control group, the marginal decreases observed in mean pup weights from group IV are not considered to be an adverse effect of treatment.

Female pups from the mid- and high-dose groups (groups III and IV) also had statistically significant decreased absolute (76 and 78% of controls', respectively) and relative (80% and 84% of controls', respectively) ovary weights at 21 days of age, but the decreases were not concentration related and were not observed at 49 days of age. In addition, decreases in ovary weights were also not observed in group VI pups.

In summary, the highest exposure level (500 ppm mixed xylene for 6 hours per day for 131 days prior to mating and continuing through lactation) in this study is interpreted as a systemic and reproductive toxicity NOAEL for adult CD rats. The study also reliably identified 500 ppm mixed xylenes as a NOAEL for maternal toxicity and developmental toxicity.

Hudák and Ungváry (1978) exposed groups of pregnant CFY rats to 0 or 230 ppm (1000 mg/m^3) xylenes (10% o-xylene, 50% m-xylene, 20% p-xylene, 20% ethyl benzene) for 24 hours per day during GDs 9–14. The exposed and control groups had 20 and 28 dams, respectively. Dams were sacrificed on GD 21, and fetuses were weighed and prepared for examination for gross, visceral, and skeletal malformations and variations. Differences between the groups were

statistically assessed with t-tests or the Mann Whitney U test, using $p<0.05$ as the level of significance. No statistically significant differences in maternal body weights; fetal deaths; mean fetal or placental weights; or external, visceral, or skeletal malformations or signs of skeletal retardation were noted in the exposed group when compared with the control group.

Exposed group fetuses showed increases for skeletal anomalies. Compared with controls, the exposed group showed statistically significant increased incidences of fetuses with fused sternebrae (7/213 vs. 1/315) and extra ribs (22/213 vs. 2/315). The incidence was based on the number of affected fetuses rather than the affected litters; litter-specific information was not provided. The interpretation of the observed statistically significant increases in the incidences of fetal skeletal anomalies (extra ribs or fused sternebrae) is confounded by the lack of ability to adjust for possible litter size covariation. The study identified 230 ppm as a NOAEL for maternal and developmental toxicity.

In a study of the potential developmental toxicity of xylene isomers (Ungváry et al., 1980), groups of 15–30 pregnant CFY rats were exposed by inhalation to air containing measured concentrations of 0, 35, 350, or 700 ppm (0, 150, 1500, or 3000 mg/m^3) of o-, m-, or p-xylene (analytical purity; actual purity not provided) for 24 hours per day on GDs 7–14. Dams were sacrificed on GD 21. Four dams in the 700 ppm m-xylene group died. Necropsy revealed hyperaemia and hemorrhage in several organs, pulmonary edema, and distention of the gut and urinary bladder. The authors stated that maternal food consumption was considerably less in the 350 and 700 ppm o-xylene or p-xylene groups during the exposure period (GDs 7–14), but returned to normal when exposure was discontinued (data were not provided).

Maternal body weight gain exhibited a concentration-related decrease during exposure to all three isomers (data not provided) but was comparable to controls' by GD 21 except for the group exposed to 700 ppm m-xylene (body weight gain 73% of controls' for GDs 0–21; $p<0.05$). Dams exposed to 350 or 700 ppm o-xylene had slightly elevated but statistically significant liver-to-body weight ratios (109 and 108% of controls', respectively) and had an increase in the rough endoplasmic reticulum profile and smooth endoplasmic vesicles as compared with controls. No other findings in the dams were reported.

Exposure to 700 ppm m-xylene—but not o- or p-xylene—resulted in a small but statistically significant decreased number of mean implantations per dam (11.44 vs. 13.52 in controls). In contrast, 700 ppm p-xylene—but not o- or m-xylene—resulted in a marked postimplantation loss (69% vs. 4% in controls) and a corresponding decreased mean litter size (8.5 vs. 12.6 in controls). Mean fetal body weights were statistically significantly decreased in the 350 and 700 ppm o-xylene groups (91 and 92% of controls', respectively) and in the 700 ppm p- and m-xylene groups (88 and 91% of the respective controls'). There was a corresponding increase in the number of weight-retarded fetuses (< 3.3 g) in these same groups.

Histochemical analyses of fetuses from the 700 ppm o- and p-xylene groups revealed decreased staining of alkaline phosphatase in the proximal convoluted tubules and of succinic hydrogenase, acid phosphatase, and glucose-6-phosphatase in the renal nephron. Additionally,

decreased activities of succinic dehydrogenase and glucose-6-phosphatase were observed in the liver and thymus cells in fetuses from the 700 ppm m-, p-, and o-xylene groups. No treatment-related changes were observed following histopathologic or electron microscopic evaluation of organs in fetuses from exposed dams.

Fetuses were examined for external, visceral, and skeletal anomalies, but only fetal incidence rates were reported; litter-specific information was not provided. No statistically significant exposure-related changes were reported for incidences of fetuses with external, visceral, or skeletal malformations in any of the groups exposed to any of the isomers. Statistically significant increases in incidence of fetuses with extra ribs were reported at 700 ppm m-xylene (8/196 [4.1%] vs. 2/284 [0.1%] for controls) or at 700 ppm p-xylene (10/51 [19.6%] vs. 6/226 [2.7%] for controls). Statistically significant increases in incidences of fetuses with skeletal retardation were also observed at 35, 350, and 700 ppm p-xylene (24/157 [15.3%], 38/197 [19.3%], and 29.4% [29.4%], respectively, vs. 16/226 [7.1%] for controls), and at 700 ppm o-xylene (48/234 [20.5%] vs. 13/168 [7.7%] for controls).

The interpretation of the observed statistically significant increases in the incidences of fetal skeletal retardation or anomalies (extra ribs) is confounded by the lack of ability to adjust for possible litter size covariation; therefore, this study identified 700 ppm o-xylene, p-xylene, or m-xylene (for 24 hours per day on GDs 7–14) as a LOAEL and 350 ppm as the highest NOAEL for maternal toxicity (decreased body weight). For p-xylene, 700 ppm is a developmental LOAEL and 350 ppm is a NOAEL for postimplantation loss, decreased litter size, and decreased fetal body weight. For m-xylene, 700 ppm and 350 ppm are a developmental LOAEL and NOAEL, respectively, for decreased fetal body weight. Finally, for o-xylene, 350 ppm is the developmental LOAEL and 35 ppm is the NOAEL for decreased fetal body weight.

In a subsequent study with rats, mice, and New Zealand rabbits, Ungváry and Tátrai (1985) exposed groups of pregnant CFY rats (19–23) to 0, 60, 440, or 780 ppm (0, 250, 1900, or 3400 mg/m^3) xylenes (presumably mixed xylenes, but the composition was not specified) for 24 hours per day during GDs 7–15. The animals were sacrificed on GD 21. Groups of pregnant CFLP mice (17–18) were exposed to 0, 115, or 230 ppm (0, 500 or 1000 mg/m^3) mixed xylenes or to 115 ppm (500 mg/m^3) o-xylene, m-xylene, or p-xylene for three 4-hour periods daily on GDs 6–15 or 7–20, respectively. Groups of 10 pregnant New Zealand rabbits were exposed to 0, 115, or 230 ppm mixed xylenes (0, 500, or 1000 mg/m^3) or 115 ppm o-, m-, or p-xylene for 24 hours per day on GDs 7–20. The mice and rabbits were sacrificed on GDs 18 and 30, respectively. Data from this study were reported in terms of percentage of fetuses affected in the four groups; litter-specific data were not reported. Maternal toxic effects were reported to be moderate and dose-dependent but were not otherwise specified.

In rats exposed to 780 ppm mixed xylenes, statistically significant findings were increased proportions of dead or resorbed fetuses (13% vs. 5% in controls), weight-retarded (not otherwise specified) fetuses (13% vs. 2%), skeletal retarded fetuses (31% vs. 13%); and fetuses with an extra rib (9% vs. 0%). In rats at 60 and 440 ppm, statistically significant findings were restricted to increased proportion skeletal retarded fetuses (32 and 33% vs. 13% in controls).

In mice exposed to mixed xylenes, statistically significant findings were restricted to the 230 ppm group (three 4-hour periods daily) and consisted of increased proportions of weight-retarded fetuses (30% vs. 7% in controls) and skeletal-retarded fetuses (13% vs. 5%). In mice exposed to 115 ppm o-, m-, or p-xylene, statistically significant findings were restricted to increased proportions of skeletal retarded fetuses (11, 11, or 12%, respectively, vs. 5% in controls).

In rabbits exposed to 230 ppm mixed xylenes for 24 hours per day, no live fetuses were produced: three dams died, six aborted, and the remaining dam showed total resorption. Another group of eight rabbits exposed to 230 ppm p-xylene for 24 hours per day also produced no live fetuses: one dam died, three aborted, and four showed total resorptions. These findings are indicative of severe maternal toxicity at this exposure level. The report did not clearly specify whether or not groups of pregnant rabbits were exposed to the o- or to the m-xylene isomers at 230 ppm. In rabbits exposed to 115 ppm mixed xylene, no increases were observed, compared with controls, in percentages of fetuses with skeletal retardation, minor anomalies, or skeletal, internal, or external malformations, but the average female (but not male) fetal body weight was statistically significantly decreased by about 10% (29.4 g vs. 32.7 g in controls). Given the small magnitude of this decrease and the inconsistency across sexes, this effect on body weight is not likely to be biologically significant.

In rabbits exposed to 115 ppm o-, m-, or p-xylene for 24 hours per day, no statistically significant differences from controls were reported in the number of abortions, number of live fetuses, proportions of dead or resorbed fetuses or fetuses with skeletal malformations, minor anomalies, or skeletal, internal, or external malformations, with the exception that an increased percentage of dead or resorbed fetuses was reported for the m-xylene groups (12.8% vs. 5.2% in controls).

As with the earlier study by Ungváry et al. (1980), interpretation of statistically significant findings for increased incidence of fetuses with retarded skeletal ossification reported by Ungváry and Tátrai (1985) is difficult, given the inability to adjust for possible litter size covariation and the relatively small magnitude of the increased incidences. For rats and mice, maternal body weight data are not reported in a manner that enables identification of maternal toxicity NOAELs or LOAELs. For rats in this study, 780 ppm mixed xylenes (for 24 hours per day on GDs 7–15) is the apparent developmental LOAEL for increased percentage of dead or resorbed fetuses, and 440 ppm is the highest NOAEL for biologically significant developmental effects. For mice, the highest exposure level—230 ppm mixed xylenes (three 4-hour periods daily on GDs 6–15)—is the apparent NOAEL for developmental effects, including skeletal, visceral, and external malformations and variations.

For mice exposed to o-, p, or m-xylene, the study identified 115 ppm (three 4-hour periods daily) as a NOAEL for biologically significant effects on fetal survival or fetal malformations or variations. For pregnant rabbits, 230 ppm mixed xylenes (for 24 hours per day on GDs 7–20) completely impaired the dams' ability to deliver live fetuses, indicating a severe maternal toxicity that was not apparent at 115 ppm; 115 ppm was also a NOAEL for

developmental toxicity. For rabbits exposed to o-, p-, or m-xylene, the study identified 115 ppm as a NOAEL for effects on fetal survival and fetal malformations or variations; exposure to p-xylene at 230 ppm produced severe maternal toxicity and no live fetuses were produced.

To evaluate the effects of prenatal exposure on postnatal neurologic development, Hass et al. (1995) exposed pregnant rats (Mol:Wist) to 0 or 500 ppm xylenes (19% o-xylene, 45% m-xylene, 20% p-xylene, 15% ethylbenzene) by inhalation for 6 hours per day on GDs 7–20 and allowed them to litter. Litter size was not standardized, but litters with fewer than six pups were not used. From each litter (n = 13–15 litters), two males and two females were kept for behavioral testing. One male and one female from each litter were kept in standardized housing until 3 months, when they underwent the Morris water maze test (finding a hidden platform while swimming in a maze). The remaining male and female from each litter were kept in enriched housing (cages contained various toys) and tested for rotarod (the ability to remain on a rotating rod for 30 seconds), open field, and Morris maze performance at about 3 months of age. The results were evaluated by analysis of variance using a repeated measures design and a significance level of p=0.05.

Exposure to xylenes did not affect maternal clinical signs, body weight gain, or food consumption. Control and exposed groups had a similar gestation period, number of pups per litter, and sex distribution per litter. The number of litters available for evaluation in the control and exposed groups was 13 and 15, respectively. Exposed litters had a slight decrease in mean birth weight (5%) and a trend toward lower body weight during the postnatal followup, but the differences did not achieve statistical significance. When the data were combined for males and females, absolute brain weights were statistically significantly decreased on postnatal day (PND) 28, but statistically significant decreases were not observed in absolute or relative brain weights when considering males or females separately or for relative brain weights of males and females combined. The air-righting reflex was statistically significantly delayed by 1 day in exposed litters due to the ability of only four pups to right themselves. No differences were observed in open field performance, and the observed decrease in rotarod performance in exposed female pups was not statistically significant.

Offspring from xylene-exposed rats that were raised in the enriched environment showed no difference in the Morris maze test when compared with controls. Offspring from exposed rats that were raised in the standard housing, however, had impaired performance. Testing at 12 weeks showed a nonstatistically significant trend (p=0.059) for increased latency for finding the platform at the beginning of the learning test. At 16 weeks, exposed offspring took statistically significantly more time to find a platform hidden in the center of the pool. Further analysis revealed that the effect was limited to the female offspring from the standard housing (not the enriched housing) and that these females had an increase in swimming length, but swim speed was unaffected. In four consecutive trials conducted at 16 weeks, the mean time (i.e., latency) to find the hidden platform was consistently greater in the standard housing exposed females than in the control female offspring.

Female offspring from the standard housing conditions were also evaluated at 28 and 52 weeks (Hass et al., 1997). At 28 weeks, an increased latency for finding a platform that was moved to a new position was observed in the female offspring only during the first trial of a three-trial testing block, whereas the next two trials resulted in similar latencies between exposed and control rats. The increased latency again corresponded with increased swimming length. No other statistically significant differences were observed for other testing situations in the Morris maze test. At 55 weeks, no statistically significant differences were observed between groups.

The Hass et al. (1995, 1997) studies found that prenatal exposure to 500 ppm xylenes, 6 hours per day on GDs 7–20 affected the performance of standard housing female rats in the Morris water maze test: it took the female offspring longer to find a hidden platform while swimming in a water maze. Although swim length (i.e., the distance covered before finding the platform) was increased, swim speed was unaffected, indicating a cognitive rather than a motor effect. This study is limited, however, in that only one concentration was tested. Overall, the results suggest that gestational exposure to 500 ppm produced a minimally adverse effect on neurologic development, and it appeared to be reversible.

The statistically significant differences between exposed and control female offspring in time to find a hidden platform that were observed at 16 and 28 weeks were not observed at 55 weeks, and the difference in latency between the exposed and control females in finding a hidden platform at 28 weeks was only observed in the first of three consecutive trials. In addition, no clear effects were observed in the other neurological tests (rotarod performance and open field activity) or in offspring housed in an enriched environment. Thus, this study identifies 500 ppm on GDs 7–20 as a developmental LOAEL for reversibly impaired Morris water maze performance in rat offspring; 500 ppm is a NOAEL for maternal toxicity.

Hass and Jakobsen (1993) exposed groups of 36 pregnant Wistar rats to air containing 0 or 200 ppm technical xylene (composition not provided) for 6 hours per day during GDs 6–20. On GD 21, two-thirds of the rats were sacrificed and were used to assess developmental toxicity. One-third of the rats were allowed to litter. Developmental milestones and rotarod performance were assessed in eight offspring (four males and four females) from each litter. No maternal toxicity was observed in the exposed dams. The only effect noted in fetuses from exposed dams was an increased incidence of delayed ossification of *os maxcillare* in the skull, with 18/26 exposed litters affected versus 2/22 control litters. In the postnatal study, statistically significantly decreased rotarod performance was observed in female pups on PNDs 22 and 23, and in male pups on PND 23.

This study is limited in that only one exposure concentration was tested and only a limited battery of behavioral tests were used. In a later report, Hass et al. (1995) questioned the significance of the effect on rotarod performance, because the testers were not blind to the exposure status of the animals. In addition, the later study did not find a clear effect of 500 ppm technical xylene (for 6 hours per day on GDs 7–20) on rotarod performance. Thus, Hass and Jakobsen (1993) did not identify a reliable developmental NOAEL or LOAEL.

Rosen et al. (1986) exposed 18 to 21 pregnant Sprague-Dawley rats to 0, 800, or 1600 ppm p-xylene (0, 3500, or 7000 mg/m^3; 99% pure) on GDs 7–16. The treatment did not affect litter size or the weight of pups at birth or on PND 3; CNS development, as measured by the acoustic startle response on PNDs 13, 17, 21, and 63, or the figure-8 maze activity, evaluated on PND 22 and 65; or the growth rate of the pups. The only effect of exposure was a statistically significant decrease in maternal body weight gain in the 1600 ppm dams (74% of controls'). The maternal toxicity LOAEL is 1600 ppm, based on decreased body weight gain, and the NOAEL is 800 ppm. The developmental neurotoxicity NOAEL is 1600 ppm.

Kükner et al. (1997/98) investigated the effect of xylene inhalation on the liver of pregnant and nonpregnant rats and pups of exposed litters by exposing pregnant Wistar rats to 0 or 2600 ppm xylenes (0 or 11,300 mg/m^3) (purity and composition not stated) for 8 hours per day on GD 6 until term (GD 21). Nonpregnant rats were exposed to 2600 ppm xylenes for the same period, and a control group of pregnant rats inhaled clean air (not stated whether nonpregnant controls were also included). Biochemical analysis of the livers from pregnant rats exposed to xylene revealed increases in AST (18%), ALT (19%), alkaline phosphatase (17%), and arginase (63%). Electron microscopic evaluation of pregnant and nonpregnant rat liver tissue revealed mitochondria that concentrated near the periphery of hepatocytes and nuclei as increased number of lysosomes and expanded smooth endoplasmic reticulum. In fetal livers from exposed litters, findings included expanded smooth endoplasmic reticulum, structurally deformed mitochondria, and granular endoplasmic reticulum. No structural defects were observed in the kidneys or pancreas of exposed pregnant or nonpregnant rats or of fetuses from exposed litters.

Mirkova et al. (1983) exposed groups of pregnant white Wistar rats to air containing 0, 3, 12, or 110 ppm (0, 14, 53, or 468 mg/m^3) xylene isomers (composition not provided) for 6 hours per day, 5 days per week, during GDs 1–21. On GD 21, a number of the animals were sacrificed for intrauterine toxicity evaluation, and the remainder were allowed to deliver for postnatal evaluations of pups. The pregnancy rates were 29/36, 11/18, 18/27, and 11/15 for the 0, 3, 12, and 110 ppm groups, respectively.

The authors reported numerous manifestations of toxicity in mid- and high-dose groups, including a statistically significant increased percentage of post-implantation loss per implantation (10.7% and 14.9%, respectively, vs. 5.5% for controls); a statistically significant decreased fetal body weight (3.20 g and 3.17 g, respectively, vs. 3.64 for controls); and a statistically significant increase in the percentage of hemorrhages in fetuses (46% and 53%, respectively, vs. 31% for controls). Although the authors reported an increased incidence of anomalies of the internal organs (including hydrocephalus, microphthalmia, intracerebral hematomas, and hemorrhages in the liver) and defects in ossification of the sternum and bones of the skull in fetuses from exposed dams, the incidence rates for these anomalies were not provided. A statistically significant decrease in pup weight on PNDs 7 and 21 was also reported for the mid- and high-dose groups, but data were not provided.

The data from this study are limited by numerous factors, including composition and purity of xylenes not being provided, incomplete description of methods, inadequate litter size

for proper fetal evaluations, high incidence of fetal hemorrhages in the control group (calling into question the health of the animals), and incomplete reporting of results. Therefore, this study does not identify reliable NOAELs or LOAELS for maternal or developmental toxicity.

4.4. OTHER STUDIES

4.4.1. Neurotoxicity Studies

4.4.1.1. Prechronic Oral Studies

In an acute study (NTP, 1986), groups of five male or five female B6C3F1 mice or Fischer 344/N rats were administered a single dose of 0, 500, 1000, 2000, 4000, or 6000 mg/kg mixed xylenes by gavage in corn oil. In mice, mortality was observed before the end of the study in 3/5 high-dose males and 4/5 high-dose females. Clinical signs reported in mice dosed with 4000 or 6000 mg/kg xylenes included tremors, prostration, and/or slowed breathing. In rats, mortality was observed within 48 hours of dosing in 5/5 high-dose males or females and in 3/5 males dosed with 4000 mg/kg. Clinical signs observed in rats dosed with 4000 or 6000 mg/kg xylenes included lack of coordination, prostration, loss of hindleg movement, and hunched posture within 24 hours of dosing, and rough coats were observed in 2000 mg/kg dose-groups. Surviving animals did not exhibit any clinical signs by the end of week 1.

In a subacute study (NTP, 1986), groups of five male or female Fischer 344 rats were dosed with 0, 125, 250, 500, 1000, or 2000 mg/kg mixed xylenes orally by gavage for 14 consecutive days. Treatment-related mortality was observed in 3/5 high-dose males and 5/5 high-dose females. High-dose male and female rats exhibited shallow labored breathing and prostration immediately after dosing. Additionally, body weight gains were reduced by 23-29% in males dosed with 250, 500, or 1000 mg/kg when compared with controls, whereas females dosed with 125 or 1000 mg/kg had body weight gains 17% and 26% lower than those of controls.

Also in NTP (1986), groups of 10 male and 10 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 17.0% ethylbenzene, 9.1% o-xylene) in corn oil by gavage at doses of 0, 125, 250, 500, 1000, or 2000 mg/kg-day for 5 days/week for 13 weeks. At termination of the study, necropsy was performed on all animals, and comprehensive histologic examinations (27 organs) were performed on vehicle and high-dose group animals. Effects noted at the high dose included the death of two female mice; clinical signs of lethargy, short and shallow breathing, unsteadiness, tremors, and paresis occurring 5–10 minutes post-dosing and lasting for 15–60 minutes in male and female mice; and decreased body weight gain in male and female mice (7% and 17%, respectively, less than controls'). No treatment-related gross or microscopic pathologic lesions were observed. No adverse effects were reported in mice dosed with 125, 250, 500, or 1000 mg/kg-day. The LOAEL is 2000 mg/kg-day, based on obvious clinical signs of nervous system depression, and the NOAEL is 1000 mg/kg-day.

4.4.1.2. Prechronic Inhalation Studies

Korsak et al. (1992) exposed groups of 12 male Wistar rats to toluene, m-xylene, or a 1:1 mixture for 6 hours per day, 5 days per week, at a concentration of 0 or100 ppm for 6 months or 1000 ppm for 3 months. Rotarod performance and spontaneous motor activity were assayed 24 hours after termination of the exposure periods. The rotarod test was used as a measure of motor coordination disturbances from exposure to m-xylene. The rotarod test involves placing the subject animals on a rotating rod and evaluating their ability to remain on the rod for a period of 2 minutes. The animals were trained to perform the task, exposed to chemical or control gas, and evaluated at defined intervals. By the time interval after exposure, considerable proportions of absorbed xylenes would be expected to have been eliminated from the body (see Section 3.4 and Appendix B). Body weights and weights of seven organs were measured; only data for animals sacrificed after 3 months of exposure was reported (controls and 1000 ppm rats).

At 3 and 6 months, blood samples were collected 24 hours after termination of exposure for measurement of serum chemistry variables (e.g., ALT, AST, sorbitol dehydrogenase, alkaline phosphatase, and total protein) and hematologic variables (erythrocyte counts, hemoglobin concentration, hematocrit, leukocyte count, and differential leukocyte counts). Serum chemistry and hematologic results were reported only for rats exposed to 1000 ppm for 3 months. Statistical evaluations (using a p=0.05 level of significance) of collected data included analysis of variance, Dunnet's test, and the Fisher exact test.

Rats exposed to m-xylene alone exhibited statistically significantly decreased rotarod performance and decreased spontaneous activity, as measured 24 hours after termination of the exposures, when compared with controls. The percentages of failures in the rotarod test were roughly 60% in rats exposed to 1000 ppm for 3 months, 35% in rats exposed to 100 ppm for 6 months, and 0% for controls at either time period. The mean spontaneous motor activity in rats exposed to 100 ppm for 6 months was about 400 movements per hour, compared with about 800 movements per hour for controls. Spontaneous motor activity data for rats exposed to 1000 ppm m-xylene for 3 months were not presented in the report.

No statistically significant exposure-related changes in body weight, absolute or relative organ weights, or clinical chemistry or hematology variables were noted in rats exposed to 1000 ppm m-xylene for 3 months, with the exception of decreased differential counts (percentage of white blood cells counted) of lymphocytes (45.5 ± 9.5 vs. 60.8 ± 6.4 for controls; 25% decrease) and increased counts of monocytes (16.3 ± 8.9 vs. 8.3 ± 4.2 for controls; 96% increase). However, total counts of white blood cells (in units of cells per mm^3 of blood) were not statistically significantly changed by exposure. The LOAEL is 100 ppm, based on decreased rotarod performance and decreased spontaneous motor activity. No NOAEL was identified.

In a second study, Korsak et al. (1994) exposed groups of 12 male Wistar rats to 0, 50, or 100 ppm m-xylene or n-butyl alcohol or a 1:1 mixture (purity of chemicals not provided) for 6 hours per day, 5 days per week, for 3 months and evaluated similar endpoints as in the earlier study. Blood for clinical biochemistry and hematologic analysis was collected 24 hours after

termination of the inhalation exposure. The report does not specify the timing of the neurologic examinations, but it appears reasonable to assume that it was the same as in the earlier study by the same investigators, that is, 24 hours after termination of exposure. Statistical evaluations (using a p=0.05 level of significance) of the collected data included analysis of variance, Dunnet's test, and the Fisher exact test.

No statistically significant exposure-related changes were noted in body weight gain, absolute or relative organ weights, hepatic activities of microsomal monooxygenases, lipid peroxidation, or levels of triglycerides in the liver. Statistically significant decreases in erythrocyte number were seen in animals exposed to 50 ppm (93% of controls') or 100 ppm (80.5% of controls') of m-xylene alone. Similarly, decreased levels of hemoglobin were reported in both groups (92% of controls' for both groups). At 100 ppm, a statistically significant increase in leukocyte number (35% increase over controls') was reported. Exposure to 50 or 100 ppm m-xylene alone also resulted in decreased rotarod performance starting at 1 month of exposure and continuing until the end of the 3-month exposure. Decreases were statistically significant in the 100 ppm group when compared with controls. The results were presented in graphical form; the actual numerical data are not provided. Decreases in performance were roughly 8% and 33% for the 50 and 100 ppm groups, respectively, versus 0% for the controls.

Sensitivity to pain was assessed using the hot plate behavior test, in which the animals are placed on a hot (54°C) surface and the time interval between being placed on the plate and licking of the paws is measured. Rats exposed to 50 or 100 ppm m-xylene alone had statistically significant increased sensitivity to pain at the end of the 3-month exposure (latency of the paw-lick response was 8.7 and 8.6 seconds, respectively, vs. 12.2 seconds for the controls). The LOAEL is 100 ppm, based on decreased rotarod performance and decreased latency in the paw-lick response in the hot-plate test, and the NOAEL is 50 ppm.

To evaluate whether xylene influences aging of the CNS or induces persistent changes in radial maze performance, Gralewicz et al. (1995) exposed 8-month-old male LOD-Wistar rats (20 per dose level) to air containing 0, 100, or 1000 ppm "pure" m-xylene (exact purity not provided) for 6 hours per day, 5 days per week, for 3 months. One-hour electroencephalograph (EEG) recordings were performed on days 28 and 56 of exposure and on days 14, 28, 56, and 84 after exposure. The number and duration of spontaneous neocortical spike and wave discharges (SWDs) from the EEG were taken as electrophysiological indices of the biological age of the brain. As rats age, SWDs increase in number and become longer. Because of large interindividual variation in number and duration of SWDs within each group, these variables were normalized to a percentage of the initial values. Exposed rats were not subjected to the daily exposure protocol when EEG recordings were made on days 28 and 56 during the exposure period.

Tests of spatial learning in an 8-arm radial maze were also conducted for a 2-week period starting from day 70 after exposure to day 83. During the first adaptation stage of the test (five consecutive daily training periods), rats were familiarized with the maze. The second stage (five

consecutive daily trials) measured effectiveness of finding water in the maze (e.g., duration of trial, number of entries into the arms, number of omission and preservation errors). One-way or two-way parametric analysis of variance was applied to the collected data, and effects were regarded as statistically significant at $p<0.05$. Body weights were also measured during and after the exposure period at various intervals, but statistically significant differences among the groups were not found.

The analysis of variance indicated no group effect on the normalized number and cumulative-duration SWD variables. However, a statistically significant group x successive recording period effect was indicated. In control rats, these variables were increased to a statistically significant degree over those in the exposed groups only on day 84 after exposure. The mean cumulative SWD duration (expressed in percentage) on day 84 was about 300 for the control, compared with means of about 150 in each of the exposed groups. The authors hypothesized that these exposure-related changes in the spontaneous, age-related changes in cortical SWD activity may have been related to cortical excitability or an increase in catecholaminergic transmissions. Unlike the controls, rats exposed to 100 or 1000 ppm m-xylene did not exhibit a statistically significant shortening of the time needed to complete a trial in the radial maze with successive daily trials.

These results indicate a learning deficit in the exposed rats. For example, on the fifth consecutive trial, the mean trial durations in each of the exposed groups were about 240–250 seconds, compared with a mean of about 150 seconds for the control group. In addition, the exposed groups did not exhibit the statistically significant decrease in omission errors with successive days in the radial arm maze test that was exhibited by the control group (number of arms in the maze omitted during a 5-minute period when the rats explored the maze). The mean number of omission errors in control rats showed a progressive decrease from about 2.75 on the first trial to 0 on the fourth and fifth successive trials. In contrast, the means on the fifth consecutive trial were about 1.5 and 2.5 for the 100- and 1000 ppm groups, respectively. The lowest exposure level in this study, 100 ppm, is designated as a LOAEL for deficits in radial maze performance.

Gralewicz and Wiaderna (2001) exposed groups of male Wistar rats (10–11 animals per group) to 0 or 100 ppm of m-xylene for 6 hours per day, 5 days per week, for 4 weeks. Behavioral testing was performed at various intervals before exposure (radial maze and open-field evaluations) and after exposure (radial maze [days 14–18], open-field activity [day 25], passive avoidance [days 39–48], hot plate test [days 50–51], and active avoidance [days 54–60]). The radial maze and hot plate test protocols were the same as in other studies by this group (Gralewicz and Wiaderna, 2001; Gralewicz et al., 1995; Korsak et al., 1992) described above.

In the open-field activity test, animals were placed in a 100 cm x 100 cm arena that was surrounded by 20 cm-high walls and divided into 49 equal squares. The number of square borders crossed (locomotor activity), number of rearings (exploratory activity), and number of grooming episodes were recorded. In the passive avoidance test, animals were placed on a platform above the floor of the cage, and the time until the animal stepped off the platform was

recorded in a series of six trials. In the first two trials, the animals were allowed to explore the cage for 60 seconds after stepping down; in the third trial, they received a series of footshocks after stepping off the platform. In trials 4, 5, and 6 the animals received no shocks and were allowed to stay on the floor for 1 minute after stepping off the platform. In the active avoidance test, the animals were trained to avoid an electric footshock by moving from one compartment of the cage to another when a sound was played. After successfully displaying avoidance behavior in four of five trials, the animals were considered to be trained. Postexposure evaluations determined the frequency of avoidance behavior in response to the same stimulus.

No differences between control and exposed rats were seen in radial maze parameters (number of arm entries, arms omitted, or arms entered multiple times) either before exposure (7 days prior to exposure) or at 14–18 days after the termination of exposure. Similarly, no differences between groups were seen in open-field activity, examined on day 8 prior to exposure and day 25 postexposure, or in active avoidance (number of trials to avoidance criterion), examined on days 54 and 60 postexposure. Xylene-exposed rats showed a significantly shorter step-down time (trial 6 only; no difference in trials 1–5) in the passive avoidance test (examined on days 39–48 postexposure) and a significantly greater paw-lick latency in the hot plate behavior test (35 seconds vs. 10 seconds in control) (examined on days 50–51 postexposure), identifying 100 ppm as a LOAEL for neurobehavioral effects.

Pryor et al. (1987) conducted studies to examine the potential for xylene to cause ototoxicity. Groups of 12 weanling male Fischer 344 rats were exposed to air containing 0, 800, 1000, or 1200 ppm mixed xylenes (10% p-xylene, 80% m-xylene, 10% o-xylene) for 14 hours per day for 6 weeks. Chamber concentrations were measured at least once daily. Hearing loss was assessed by measuring behavioral auditory thresholds (conditioned avoidance response task), whereby rats were trained to pull or climb a pole suspended from the ceiling to avoid a shock following warning tones and by measuring brainstem auditory-evoked response (BAER), an electrophysiologic measurement of auditory function. The frequencies of the tones tested were 4, 8, 12, and 20 kHz, with the sound levels (decibels) varying with frequency. Results were presented only in graphical form, with actual data not provided. Tests were conducted every 2 weeks during exposure and at 2 weeks postexposure.

All xylene-exposed groups had dose-dependent increases in behavioral auditory and BAER thresholds relative to controls at some frequencies. Behavioral auditory thresholds were elevated at 12 and 20 kHz in 800 ppm-group rats; at 8, 12, and 20 kHz in 1000 ppm-group rats; and at all frequencies in 1200 ppm-group rats. BAER thresholds were elevated at 16 kHz in 800 ppm-group rats; at 8 and 16 kHz in 1000 ppm-group rats, and at 4, 8, and 16 kHz in 1200 ppm-group rats (8kHz not tested for BAER threshold determinations). No other indices of toxicity were investigated. On the basis of increased behavioral auditory and BAER thresholds, the LOAEL is 800 ppm and the NOAEL is not determined.

Nylén and Hagman (1994) exposed groups of male Sprague-Dawley rats by inhalation to air containing 0, or 1000 ppm xylene (1.5% o-xylene, 65% m-xylene, 32% p-xylene, 2.5% ethylbenzene), 1000 ppm n-hexane, or 1000 ppm of n-hexane mixed with xylene, for 18 hours

per day, 7 days per week, for 61 days. Two days following exposure, rats exposed to xylene alone had statistically significantly decreased body weights and a slight loss in auditory sensitivity, as recorded by auditory brainstem response, compared with controls. Xylene exposure did not affect flash-evoked potentials or nerve and muscle action potentials measured in the tail.

As discussed in Section 4.2.1.2. (Jenkins et al., 1970), one of two dogs exposed to 780 ppm o-xylene for 8 hours per day, 5 days per week, for 6 weeks exhibited tremors throughout the exposure. No additional information was provided.

In a study conducted by Savolainen et al. (1979), groups of 20 male Wistar rats were exposed to vapors containing 300 ppm xylenes (85% m-xylene, 15% o- and p-xylene) or control air for 6 hours per day, 5 days per week, for 5 to 18 weeks with or without concomitant exposure to ethanol in drinking water. Exposure to xylene alone resulted in an increase in microsomal superoxide dismutase activity in the brain at the end of the exposure and transient decreased preening frequency.

Rosengren et al. (1986) exposed groups of four male and four female Mongolian gerbils by continuous inhalation to xylene at 0, 160, or 320 ppm for a period of 3 months, followed by a 4-month postexposure solvent-free period. Xylene exposure caused regional increases in the brain concentrations of glial fibrillary acidic protein, a main component of astroglial filaments; S-100 protein (found in fibrillary astrocytes); and DNA. The authors stated that these findings were compatible with the presence of astrogliosis. No other evaluations, including a recording of clinical signs, were described.

Studies have also been conducted to assess the potential for xylene exposure in utero to result in postnatal neurobehavioral deficits. For more information on these studies, the reader is referred to Section 4.3.2.

4.4.2. Genotoxicity

The genotoxicity of commercial xylenes and all three individual isomers has been studied, and the results are, for the most part, negative (IARC, 1989). All studies cited in the GENE-TOX data base are negative with the exception of one study for which no conclusion was drawn. Xylenes are not mutagenic in bacterial test systems with *Salmonella typhimurium* (Bos et al., 1981; Florin et al., 1980; NTP, 1986) and *Escherichia coli* (McCarroll et al., 1981) or in cultured mouse lymphoma cells (Litton Bionetics, 1978b). Xylenes do not induce chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary cells (Anderson et al., 1990) or cultured human lymphocytes (Gerner-Smidt and Friedrich, 1978), chromosomal aberrations in rat bone marrow (Litton Bionetics, 1978b), micronuclei in mouse bone marrow (Mohtashamipur et al., 1985), or sperm head abnormalities in rats (Washington et al., 1983). Technical grade xylenes—but not o- and m-xylene—are weakly mutagenic in *Drosophila* recessive lethal tests (Donner et al., 1980). No increase in the frequency of sister chromatid exchanges was observed

in peripheral lymphocytes in individuals exposed to xylenes in an occupational setting (Haglund et al., 1980; Pap and Varga, 1987) or an experimental setting (Richer et al., 1993).

4.4.3. Comparison of the Toxicity of Individual Xylene Isomers

Technical-grade mixed xylenes, the form most commonly used as a commercial solvent, is a blend of three isomers (o-, m-, and p-xylene), and it frequently contains a significant portion of ethylbenzene. Humans are most likely exposed to a mixture of xylenes rather than to individual isomers, and it is the solvent used most frequently in toxicity studies. It should be noted, however, that the composition of the mixture (relative amounts of the individual isomers and ethylbenzene) varies considerably depending on its source. Results from studies comparing the toxicity of individual xylene isomers indicate that differences, when they occur, are specific to the endpoint under consideration.

For oral exposure, minimal comparison studies on the toxicity of individual xylene isomers are available. Condie et al. (1988) reported no significant differences in body weight changes in male Sprague Dawley rats given 10 consecutive oral doses of 2000 mg/kg-day of each of the individual isomers. Furthermore, although the 90-day studies by Wolfe et al. (1988a, b) reported decreased body weights at 800 mg/kg-day of both m-xylene and p-xylene, Condie et al. (1988) did not report decreased body weight at 750 mg/kg-day when mixed xylenes were administered to the same strain of rats for 90 days.

For inhalation exposure, various patterns of toxicity among xylene isomers have been reported. In rats exposed by inhalation for 30 minutes, EC_{50}s (the concentrations producing half-maximal decreases in response rate) for effects on an operant behavior test showed a relative toxicity order of o-xylene > p-xylene > m-xylene, whereas EC_{50}s for a motor performance test showed a toxicity order of p-xylene > o-xylene = m-xylene. The range of EC_{50} values among the isomers was not considered large (Moser et al., 1985). In contrast, p-xylene—but not o-xylene or m-xylene—altered audiometric variables in rats exposed to 1800 ppm 6 hours per day, 5 days per week, for 13 weeks (Gagnaire et al., 2001).

A different order of toxicity has been described for effects on motor coordination (rotarod performance) in rats following 6 hours exposure to concentrations of 3000 ppm xylenes: o-xylene > m-xylene > p-xylene (Korsak et al., 1990). All three isomers caused decreased fetal body weights in rats exposed for 24 hours per day on GDs 7–14, but o-xylene caused the effect at a lower concentration (350 ppm) than did either p-xylene or m-xylene (700 ppm) (Ungváry et al., 1980). No available studies compare the potency of isomers for affecting neurological endpoints following subchronic or chronic inhalation exposure.

Some of the studies that have examined the toxicity of individual xylene isomers are described in more detail below.

Condie et al. (1988) did not find any significant differences in the toxicity of the individual isomers in an experiment in which groups of 10 male and 10 female Sprague-Dawley rats were administered m-, o-, or p-xylene orally by gavage in corn oil for 10 consecutive days at doses of 0, 250, 1000, or 2000 mg/kg-day. Two female rats receiving the high dose of p-xylene died; the deaths were attributed to treatment. Male rats receiving 2000 mg/kg-day of each isomer had statistically lower body weights (88–94% of controls'), whereas body weights of high-dose females were not affected. Males and females receiving 2000 mg/kg-day of each isomer had statistically elevated liver weights and/or liver to body weight ratios (ranging from 128 to 148% of controls'). The authors concluded that there are no significant differences in the toxicity of the individual isomers.

Moser et al. (1985) evaluated the effects of the individual xylene isomers and a commercial xylene mixture on operant responding and motor performance in CD-1 male albino mice following 30-minute static inhalation exposures. The minimally effective concentration for disruption of operant performance (lever-pressing behavior) was 1400 ppm for all isomers, with an EC_{50} of 6200, 5200, or 5600 ppm for m-xylene, o-xylene, and p-xylene, respectively. The minimally effective concentrations for the inverted screen test (mice falling off the screen or unable to travel the 13 cm distance in 60 seconds) were 3000 ppm for m-xylene and o-xylene and 2000 ppm for p-xylene, and the EC_{50} values for performance on the inverted screen test were 3790, 3640, and 2676 ppm for m-xylene, o-xylene, and p-xylene, respectively. There were no consistent, significant differences in the potency of the individual isomers. Although o-xylene exhibited a more potent effect on operant behavior, p-xylene more severely affected motor performance.

Gagnaire et al. (2001) exposed groups of male Sprague Dawley rats (n = 16) to 0, 450, 900, or 1800 ppm of o-, m-, or p-xylene for 6 hours per day, 5 days per week, for 13 weeks. Audiometric measurements were made using implanted electrodes at weeks 0, 4, 8, and 13 of exposure and at weeks 4 and 8 postexposure. Neither o-xylene or m-xylene resulted in detectable changes in audiometric measurements, either during exposure or during the 8 week postexposure recovery period. In contrast, exposure to 1800 ppm of p-xylene caused significant decrements in brainstem auditory-evoked potential (2, 4, 8, and 16 KHz) and cochleograms (total cell count) at every time point examined. No changes were seen in animals exposed to 450 or 900 ppm at any time examined.

In a study by Molnár et al. (1986), motility was assessed in groups of eight CFY white male rats following exposure by inhalation for 4 hours to at least six concentrations each of m-xylene, o-xylene, or p-xylene (individual concentrations not provided). Exposure to 130 to 1500 ppm m-xylene and 400 to 1500 ppm p-xylene resulted in a dose-dependent increase in group motility, whereas exposure to 150 to 1800 ppm o-xylene resulted in a slight depression of activity. At higher concentrations, however, activity was decreased in all groups, with the minimum narcotic concentration for the three isomers reported as 2180 ppm for o-xylene, 2100 ppm for m-xylene, and 1940 ppm for p-xylene.

Korsak et al. (1990) found that o-xylene, in comparison with other isomers, more severely affected motor performance. Groups of 10 male Wistar rats were exposed to approximately 3000 ppm o-, m-, or p-xylene for 6 hours, with rotarod performance measured before exposure and immediately after termination of the exposure. The results of the testing, given in terms of the number of failures per number of tested animals, were as follows: for 19/20 for o-xylene at an average concentration of 3027 ppm, 6/20 for m-xylene at an average concentration of 3093 ppm, and 1/20 for p-xylene at an average concentration of 3065 ppm.

To address the potential for xylene isomers to cause maternal or developmental toxicity, Ungváry et al. (1980) exposed groups of 15–30 pregnant CFY rats to air containing measured concentrations of 35, 350, or 700 ppm of o-, m-, or p-xylene continuously during GDs 7–14. Dams were sacrificed on GD 21. The general conclusion of the study was that exposure to m-xylene was the most toxic to the dams, whereas fetal toxicity varied with the isomer. m-Xylene exposure (700 ppm) resulted in a decreased number of mean implantations per dam. p-Xylene exposure (700 ppm) resulted in increased post-implantation loss and a correspondingly decreased litter size. All concentrations of p-xylene and the highest concentration of o-xylene resulted in increased fetal incidence of skeletal retardation. Finally, all isomers caused decreased fetal body weight: o-xylene at 350 and 700 ppm and m-xylene and p-xylene at 700 ppm.

Fang et al. (1996) determined the Minimum Alveolar Concentration (MAC) (the concentration that produces anesthesia, i.e., lack of movement, in 50% of those exposed) of the individual isomers in rats. The MACs of o-, m-, and p-xylene were 0.00118 ± 0.00009, 0.00139 ± 0.00010, and 00.00151 ± 0.0007 atm, respectively, with a difference in MAC values of less than 30% among the isomers.

In summary, although differences in the toxicity of the xylene isomers have been detected, no consistent pattern following oral or inhalation exposure has been identified.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

4.5.1. Oral Exposure

Human studies following oral exposure to xylenes are not available. Results from several subchronic studies in rats and mice (Condie et al., 1988; NTP, 1986; Wolfe et al., 1988a, b) and one chronic study in male rats (NTP, 1986) identify decreased body weight as a potential health hazard from repeated oral exposure to dose levels generally greater than 500–800 mg/kg-day. Xylene-induced body weight decreases have been observed most consistently in male rats (Condie et al., 1988; NTP, 1986; Wolfe et al., 1988a, b). There have been observations of mild body weight decreases in female rats exposed for 90 days to 800 mg/kg-day m-xylene or p-xylene (Wolfe et al., 1988a, b). Decreased body weights (93% of controls') were also observed in mice exposed for 13 weeks to 2000 mg mixed xylenes/kg-day (NTP, 1986). Decreased body weights were not observed in female rats or in male and female mice exposed for 2 years to

doses of xylenes of up to 500 or 1000 mg/kg-day, respectively (NTP, 1986). The mode of action responsible for this effect has not been studied.

Studies of orally exposed animals provide some evidence for other noncancer effects at a few sites, but these appear to occur either at dose levels above the lowest levels inducing body weight changes or inconsistently across studies. Several subchronic studies (Condie et al., 1988; Wolfe et al., 1988a, b) reported nonneoplastic and organ weight findings. Increased liver weights, in the absence of histopathologic changes, were observed in one study (Condie et al., 1988) in male and female rats exposed for 90 days to mixed xylenes at doses $\geq$150 and 750 mg/kg-day, respectively. However, similar liver weight changes were not observed in other subchronic studies of rats (NTP, 1986; Wolfe et al., 1988a, b) and mice (NTP, 1986) exposed to doses as high as 1000 and 2000 mg/kg-day, respectively, or in the only available chronic study of rats and mice exposed to doses as high as 500 and 1000 mg/kg-day, respectively (NTP, 1986). Increased kidney weights, with associated signs of nephropathy, were observed in female but not male rats exposed for 90 days to mixed xylenes (Condie et al., 1988), but similar kidney effects were not observed in the other subchronic studies (NTP, 1986; Wolfe et al., 1988a, b).

Neurological effects from oral exposure to xylenes are a consideration, based on the reports of neurological symptoms in workers exposed to airborne xylenes. Animal data on oral exposure to support this assumption are limited and indicate that gross clinical signs and symptoms of neurological impairment occur only at exposure levels above the lowest levels associated with body weight changes. In 13-week studies (NTP, 1986), weakness, lethargy, short and shallow breathing, unsteadiness, tremors, and paresis were observed in mice exposed to 2000 mg/kg-day mixed xylenes. These symptoms were observed 5–10 minutes after dosing and lasted for 15–60 minutes. Similar transient neurological symptoms were not observed or were not reported in mice at lower exposure levels or in rats exposed to 1000 mg/kg-day. In 2-year studies (NTP, 1986), hyperactivity 5–30 minutes after dose administration was observed in mice at 1000 mg/kg-day, but not at 500 mg/kg-day. Other subchronic rat studies (Condie et al., 1988; Wolfe et al., 1988a, b) reported no gross clinical or histological findings indicative of neurological impairment. The oral data base is limited in that comprehensive examinations of persistent changes in neurobehavior (e.g., a functional observational battery) following acute or repeated oral exposure to xylenes have not been conducted.

Results from two animal studies indicate that developmental effects are a potential hazard from oral exposure to xylenes, but the effects occur at dose levels greater than those inducing body weight changes. Cleft palate formation was reported in the fetuses of mice exposed to 2060 mg/kg-day—but not 1030 mg/kg-day—on GDs 6–15 (Marks et al., 1982). Another gestational exposure study of mice (Nawrot and Staples, 1980) (reported only as an abstract) also reported cleft palate formation at 1960 mg/kg-day but not at 780 mg/kg-day. Maternal toxicity was not sufficiently evaluated in either of these studies to identify maternal NOAELs and LOAELs. No available studies have examined the potential effects of oral exposure of xylenes on reproductive endpoints.

4.5.2. Inhalation Exposure

The weight of evidence from limited human data and more extensive animal data indicates neurological impairment and developmental effects as potential health hazards from repeated inhalation exposure to xylenes. Reversible symptoms of neurological impairment and irritation of the eyes and throat are well-known health hazards from acute inhalation exposure to xylenes and other aromatic solvents. In general, these acute effects are expected to involve reversible molecular interactions of the solvent itself (not metabolites) with membranes of the affected tissues, including neuronal membranes, and are most pronounced at high exposure levels (in excess of 1000 ppm). At lower concentrations, more subtle effects may occur. Human volunteers exposed under controlled conditions to xylene concentrations in the range of 200–400 ppm for short time periods (15 minutes to 4 hours) reported symptoms of irritation (e.g., watering eyes and sore throat) or neurological impairment (e.g., mild nausea, headache) (Carpenter et al., 1975; Gamberale et al., 1978).

In other studies involving single or multiple 4-hour exposures of human volunteers to 200 ppm xylene (Laine et al., 1993; Savolainen and Linnavuo, 1979; Savolainen et al., 1984), reversible effects on balance and reaction times were reported. However, other studies of 4-hour exposures to 200 ppm did not find impaired performance in tests of simple reaction time, short-term memory, and choice reaction time (Olson et al. 1985) or changes in visually evoked brain potentials (Seppäläinen et al., 1983) or EEG patterns (Seppäläinen et al., 1991). Impaired performance on tests of memory and reaction times was reported for subjects exposed to 100 ppm xylene for 4 hours (Dudek et al., 1990).

The available controlled-exposure human studies indicate that concentrations around 100–200 ppm are close to the threshold level for short-term reversible neurological and irritation effects from xylenes. Available human data alone provide inadequate evidence for neurological impairment from repeated exposure to xylene concentrations less than or equal to 200 ppm. Aside from the controlled-exposure studies reviewed above, most of the human data associating xylene exposure with neurological impairment are case reports involving acute high-level exposures (800–10,000 ppm) (e.g., Goldie, 1960; Hipolito, 1980; Klaucke et al., 1982).

Epidemiologic studies are restricted to a cross-sectional health evaluation study (Uchida et al., 1993) that noted increased prevalence of self-reported neurological symptoms and irritation—but no apparent changes in serum enzymes indicative of liver or kidney damage—in a group of Chinese workers. The workers were from a boot manufacturing plant that used a xylene-containing glue and two other plants that used mixed xylenes as a solvent in wire production or printing. The measured time-weighted-average (TWA) mean concentration of airborne xylenes in these workplaces was 21 (± 21) ppm. The study has several limitations, including a lack of reporting on the duration of exposure, co-exposure to other chemicals, no clear demonstration of relationships between response and dose or duration, and the inherent bias presented by self-reporting of symptoms.

Although the human evidence for persistent effects on the nervous system or other persistent effects from repeated inhalation exposure to xylenes is inadequate, results from animal studies

more clearly identify potential persistent neurological impairment and possible developmental effects as potential health hazards from repeated inhalation exposure.

A number of subchronic studies in animals provide evidence for neurological effects following repeated inhalation exposure to xylenes (Table 2). The lowest exposure level that produced changes in a number of neurological endpoints was identified in several studies of rats exposed to 100 ppm m-xylene, 6 hours per day, 5 days per week, for 3 months. These studies observed statistically significant changes in neurobehavioral tests conducted at least 24 hours following cessation of exposure: decreased rotarod performance indicative of impaired motor coordination (Korsak et al., 1992, 1994), decreased spontaneous motor activity (Korsak et al., 1992), impaired radial maze performance indicative of a learning deficit (Gralewicz et al., 1995), and decreased latency to paw lick in the hot plate test, indicating increased sensitivity to pain (Korsak et al., 1994).

At a lower exposure level (50 ppm by the same exposure protocol), rats showed statistically significantly decreased latency in the paw lick response but no statistically significant effects on rotarod performance (Korsak et al., 1994). Rats exposed to 100 ppm m-xylene by the same daily protocol for a shorter duration (4 weeks) displayed no statistically significant changes in tests of radial maze performance, open-field activity, or active avoidance, but paw lick latency was increased in the hot plate test and step-down time was shortened in 1/6 trials in the passive avoidance test (Gralewicz and Wiaderna, 2001).

Table 2. Neurological effects of xylenes following subchronic inhalation exposure of adult male rats

Study	Duration	NOAEL (ppm)	LOAEL (ppm)	Effect
Korsak et al. (1994)	6 hrs/day, 5 days/wk, 3 months	50	100	Impaired rotarod performance, decreased latency of paw-lick response
Korsak et al. (1992)	6 hrs/day, 5 days/wk, 6 months	None	100	Impaired rotarod performance, decreased motor activity

Gralewicz et al. (1995)	6 hrs/day, 5 days/wk, 3 months	None	100	Altered radial maze performance
Gralewicz and Wiaderna (2001)	6 hrs/day, 5 days/wk, 4 weeks	None	100	Altered passive avoidance test, increased latency of paw-lick response
Pryor et al. (1987)	7 hrs/day, 7 days/wk, 6 weeks	None	800	Hearing loss
Nylén and Hagman (1994)	18 hrs/day, 7 days/wk, 61 days	None	1000	Decreased auditory sensitivity

Overall results from these rat studies provide evidence that repeated exposure to m-xylene at concentrations ≥100 ppm (6 hours per day, 5 days per week) may produce persistent changes in several neurologic endpoints in adult rats. The mode of action of these changes has not been studied. Supporting evidence for persistent neurologic effects from xylenes exposure includes reports of changes in indices of hearing loss in rats exposed to ≥800 ppm mixed xylenes 14 hours per day for 6 weeks (Pryor et al., 1987) and in rats exposed to 1000 ppm mixed xylenes 18 hours per day, 7 days per week, for 61 days (Nylén and Hagman, 1994).

There are no available studies on the possible developmental toxicity of inhaled xylenes in humans, but a number of studies have examined standard developmental toxicity endpoints and neurobehavioral endpoints in offspring of animals exposed to mixed xylenes or individual xylene isomers. Pertinent developmental effect levels in animals exposed by inhalation to xylenes during gestation are summarized in Table 3.

Table 3. Pertinent developmental effect levels in animals exposed by inhalation to xylenes during gestation

Species	Effect/Endpoint	NOAEL (ppm)	LOAEL (ppm)	Exposure	Reference
Rat	Impaired cognitive (but not motor) performance in Morris water maze by female (but not male) offspring at 500 ppm	None identified	500	0, 500 ppm mixed xylenes, 6 hrs/day, GDs 7–20	Hass et al. (1995, 1997)
	No effects on rotarod performance by offspring at 500 ppm	500	None identified	0, 500 ppm mixed xylene, 6 hrs/day, GDs 7–20	Hass et al. (1995)
	No effects on acoustic startle response or figure-8 maze activity in offspring, PNDs 22 and 65 at concentrations up to 1600 ppm	1600	None identified	0, 800, 1600 ppm p-xylene, 6 hrs/day, GDs 7–16	Rosen et al. (1986)
	Effects on fetal skeletal and visceral malformations or variations, fetal BW, or maternal BW gain at concentrations up to 400 ppm[a]	400	None identified	0, 100, 400 ppm mixed xylenes 6 hrs/day, GDs 6–15	Litton Bionetics (1978a)
	No effects on fetal skeletal and visceral malformations or variations, fetal BW, maternal BW, or maternal fertility at 500 ppm[a]	500	None identified	0, 500 ppm mixed xylenes 6 hrs/day for 131 days prior to mating and continuing to GD 21or through lactation	Bio/dynamics Inc. (1983)
	Decreased maternal BW gain at 1600 ppm; no effects on litter size, pup birth weight, or postnatal growth rate	800	1600	0, 800, 1600 ppm p-xylene, 6 hrs/day, GDs 7–16	Rosen et al. (1986)
	Maternal toxicity (decreased BW) at 700 but not 350 ppm; decreased fetal BW at 350 and 700 ppm but not 35 ppm; effects on fetal skeletal and visceral malformations or variations[a]	35	350	0, 35, 350, 700 ppm o-xylene, 24 hrs/day GDs 7–14	Ungváry et al. (1980)
	Maternal toxicity (decreased BW) at 700 but not 350 ppm; decreased fetal BW at 700 ppm but not 350 or 35 ppm; effects on fetal skeletal and visceral malformations or variations[a]	350	700	0, 35, 350, 700 ppm m-xylene, 24 hrs/day GDs 7–14	Ungváry et al. (1980)
	Maternal toxicity (decreased BW) at 700 but not 350 ppm; post-implantation loss, decreased litter size, and decreased fetal BW at 700 ppm but not 350 or 35 ppm; effects on fetal skeletal and visceral malformations or variations[a]	350	700	0, 35, 350, 700 ppm p-xylene, 24 hrs/day GDs 7–14	Ungváry et al. (1980)

Table 3. Pertinent developmental effect levels in animals exposed by inhalation to xylenes during gestation (continued)

Species	Effect/Endpoint	NOAEL (ppm)	LOAEL (ppm)	Exposure	Reference
Rat (cont'd)	Maternal toxicity not clearly reported; increased proportion dead or resorbed fetuses at 780 ppm; effects on fetal skeletal and visceral malformations or variations[a]	440	780 ppm	0, 60, 440, 780 ppm mixed xylenes, 24 hrs/day GDs 7–15	Ungváry and Tátrai (1985)
Mouse	Maternal toxicity not clearly reported; effects on fetal skeletal or visceral malformations or variations[a]	230	None identified	0, 115, 230 ppm mixed xylenes, three 4-hour periods daily on GDs 6–15	Ungváry and Tátrai (1985)
	Maternal toxicity not clearly reported; effects on fetal skeletal or visceral malformations or variations[a]	115	None identified	0, 115 ppm o-, p-, or m-xylene, three 4-hour periods daily on GDs 6–15	Ungváry and Tátrai (1985)
Rabbit	No live fetuses produced at 230 ppm and three dams died, indicating severe maternal toxicity; no statistically significant effects on abortions, live fetus numbers, or fetal skeletal or visceral malformations or variations at 115 ppm; mean female (but not male) fetal BW decreased by about 10% when compared with controls at 115 ppm	None identified	115 (body weight) 230 (dead fetuses)	0, 115, 230 ppm mixed xylenes, 24 hrs/day GDs 7–20	Ungváry and Tátrai (1985)
	No effects on fetal survival or fetal skeletal or visceral malformations or variations at 115 ppm[a] ; at 230 ppm p-xylene, no live fetuses produced and one dam died, indicating severe maternal toxicity; report did not specify whether rabbits were exposed to o- or m-xylene at 230 ppm	115	230	0, 115 ppm o-, p, or m-xylene, 24 hrs/day GDs 7–20; 230 ppm p-xylene 24 hrs/day GDs 7–20	Ungváry and Tátrai (1985)

[a] Statistically significant increases in incidences of fetuses with retarded skeletal ossification or with extra ribs were reported as compared with controls. However, for all of the studies except Litton Bionetics (1978a), the incidences were reported on a total-fetus-per-exposure-group basis; no litter-specific data were reported. These effects were judged to be difficult to interpret, given the inability to adjust for possible litter size covariation. For example, after adjustment for covariance with litter size, the incidences of fetuses with delayed ossification in rats exposed to 400 ppm 6 hrs/day in Litton Bionetics (1978a) were no longer significant. The statistically significantly elevated incidences of skeletal anomalies or retardation were not considered adverse in determining developmental NOAELs and LOAELs in these studies. See Section 4.3.2 for more discussion.

Table 3. Pertinent developmental effect levels in animals exposed by inhalation to xylenes during gestation (continued)

PND = postnatal day
BW = body weight

Evidence exists for impaired neurological development in rat offspring following gestational exposure to inhaled xylenes, but it is not strong. Changes in neurobehavioral variables reported for offspring of animals exposed during gestation are restricted to impaired cognitive (but not motor) performance in the Morris water maze test in female but not male offspring of rats exposed to 500 ppm mixed xylenes 6 hours per day on GDs 7–20 (Hass et al., 1995, 1997) and decreased rotarod performance in offspring of rats exposed to 200 ppm "technical" xylene 6 hours per day on GDs 6–20 (Hass and Jakobsen, 1993). Deficits in the water maze test were observed only in female rat offspring raised in standard housing and not in female rats raised in "enriched" housing with various toys (Hass et al., 1995).

Although decreased rotarod performance by offspring was observed in the study by Hass and Jakobsen (1993), it was not observed in the later study by the same group of investigators (Hass et al., 1995). The reported effect on rotarod performance in the earlier study was questioned by Hass et al. (1995) because it was not conducted by experimenters who were blind to the exposure status of the rats. In addition, offspring of rats exposed to 800 or 1600 ppm p-xylene, 6 hours per day on GDs 7–16 performed similarly to offspring of nonexposed rats in tests of CNS development—an acoustic startle response test on PNDs 13, 17, 21, and 63 and a figure-8 maze activity test on PNDs 22 and 65 (Rosen et al., 1986).

Several other inhalation studies have examined standard developmental toxicity endpoints in rats (Litton Bionetics, 1978a; Bio/dynamics Inc., 1983; Rosen et al., 1986; Ungváry et al., 1980; Ungváry and Tátrai, 1985) and mice and rabbits (Ungváry and Tátrai, 1985) following gestational exposure to xylenes (Table 3). These studies identified maternally toxic levels for decreased body weight gain in pregnant rats at doses greater than or equal to 700 ppm o-, p-, or m-xylene 24 hours per day (Ungváry et al., 1980) or 1600 ppm p-xylene 6 hours per day (Rosen et al., 1986) and for maternal death and abortions in pregnant rabbits exposed to 230 ppm (but not 115 ppm) mixed xylenes or p-xylene for 24 hours per day (Ungváry and Tátrai, 1985).

In rats, effects on fetal skeletal and visceral malformations (such as cleft palate) and variations (such as retarded skeletal ossification or extra ribs) were reported at concentrations of up to 700 ppm o-, m-, or p-xylene 24 hours per day (Ungváry et al., 1980) or 780 ppm mixed xylenes 24 hours per day. Statistically significant increased incidences of fetuses with retarded skeletal ossification or extra ribs were reported in these studies, but on an exposure-group basis. No litter-specific information was provided except in the Litton Bionetics (1978a) study.

The most significant effects on developmental endpoints were decreased fetal body weight or fetal survival in rats at xylene isomer doses of 350 or 700 ppm 24 hours per day (Ungváry et al., 1980) or at mixed xylenes concentration of 780 ppm 24 hours per day (Ungváry and Tátrai, 1985) and increased abortions in rabbits exposed to 230 ppm 24 hours per day (Ungváry and Tátrai, 1985). These effects, although of concern, occurred at concentrations above those at which neurobehavioral effects were reported in adult animals (see Table 2).

Although the mechanism by which xylenes exert their toxic effects on the nervous system and developing fetus are not completely understood, some theories exist. The CNS toxicity

observed during exposure to high concentrations has been attributed to the liposolubility of xylenes in the neuronal membrane (Desi et al., 1967; Savolainen and Pfaffli, 1980; Tahti, 1992). It has been suggested that xylenes disturb the actions of proteins essential to normal neuronal function. Changes in levels of various neurotransmitters and lipid composition have been observed in several brain areas following acute- and intermediate-duration exposure to xylenes (Andersson et al., 1981; Honma et al., 1983). It is unclear whether these represent direct effects of xylenes or are secondary changes resulting from nonspecific CNS depression. Some authors have also suggested that metabolic intermediates such as arene oxides or methylbenzaldehyde may be responsible for the toxic effects of xylenes (Savolainen and Pfaffli, 1980).

The mechanism by which xylenes produce toxic effects in fetuses has not been fully investigated. The p-xylene-induced delayed fetal development found in studies with rats (Ungváry et al., 1981) may have been caused by decreased levels of progesterone and estradiol. The decreased hormonal levels may have been due to increased microsomal activity and increased hormonal catabolism.

In summary, human data are suggestive of neurological effects and irritation of the eyes and respiratory tract following inhalation exposure to xylenes. Animal studies have demonstrated that neurological effects are the most sensitive effects of xylenes inhalation exposure, with measurable effects in several neurobehavioral endpoints beginning at concentrations as low as 100 ppm following subchronic exposure (Gralewicz et al., 1995; Korsak et al., 1992, 1994; Nylén and Hagman, 1994; Pryor et al., 1987). At higher exposure levels, changes in body weight have been reported in some studies (Tátrai and Ungváry, 1980; Tátrai et al., 1981) but not in others (Carpenter et al., 1975; Jenkins et al., 1970; Ungváry, 1990).

Similarly, high-dose exposure to xylenes has resulted in changes in liver morphology, weight, and enzymatic functions (Tátrai and Ungváry, 1980; Tátrai et al., 1981; Ungváry, 1990). Gestational exposure of animals to xylenes has resulted in neurodevelopmental effects (Hass et al., 1995; 1997; Hass and Jakobsen, 1993) and other possible developmental effects (Ungváry et al., 1980; Ungváry and Tátrai, 1985), but only at levels above those at which neurobehavioral effects in adult male rats were reported.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999) *data are inadequate for an assessment of the carcinogenic potential* of xylenes. Adequate human data on the carcinogenicity of xylenes are not available, and the available animal data are inconclusive as to the ability of xylenes to cause a carcinogenic response. Evaluations of the genotoxic effects of xylenes have consistently provided negative results.

Data on the carcinogenicity of xylenes following inhalation exposure are limited. A number of human occupational studies have suggested possible carcinogenic effects of chronic inhalation exposure. However, in each case co-exposure to other chemicals was a major confounder,

leading to an inability to adequately assess the potential effects of chronic exposure. Animal data on the carcinogenicity of xylenes following inhalation exposure are not available.

Examinations of the carcinogenicity of xylenes following oral exposure in humans are not available. NTP (1986) conducted a 2-year oral cancer bioassay in male and female Fischer 344 rats and male and female B6C3F1 mice. Rats were exposed to 0, 250, or 500 mg/kg-day of mixed xylenes by gavage for 5 days per week for 103 weeks. No evidence of carcinogenesis was seen in male or female rats. Similarly, mice exposed to 0, 500, or 1000 mg/kg-day for 2 years did not show evidence of carcinogenic effects (NTP, 1986). However, a study by Maltoni et al. (1983, 1985) reported an increase in the overall number of malignant tumors in male and female rats treated by gavage with 0 or 500 mg/kg mixed xylenes for 4 days per week for 104 weeks. However, only total tumor incidence was reported; descriptions of target organs and tumor types were not included in the report. In the absence of additional information, and because only one dose was used, the Maltoni et al. (1983, 1985) study does not provide sufficient evidence of the carcinogenicity of xylenes in animals.

4.7. SUSCEPTIBLE POPULATIONS

No definitive data addressing susceptible populations are available.

Available human data are not adequate to determine whether a gender-related difference in susceptibility to xylene exists. Differences in sensitivity between males and females in animal studies have been inconsistent across species or endpoint. For example, male but not female rats consistently showed decreased body weight changes following repeated oral exposure to mixed xylenes in several studies (NTP, 1986; Condie et al., 1988), whereas body weight changes were observed in both male and female mice exposed to 2000 mg/kg/day mixed xylenes for 13 weeks (NTP, 1986). In contrast, female but not male offspring of rats exposed by inhalation to 500 ppm mixed xylenes 6 hours per day on GDs 7–20 showed reversible impairment of learning in the Morris water maze test (Hass et al., 1995; 1997). However, the adversity of this effect appears to be minimal, because only one group of female offspring (those housed under standard conditions without various toys) showed the effect and the impairment appeared to diminish with time. In addition, other neurological endpoints (acoustic startle response or figure-8 maze activity) were not affected in either male or female offspring of rats exposed to up to 1600 ppm p-toluene 6 hours per day on GDs 6–15 (Rosen et al., 1986).

For other relevant endpoints, possible differences between genders have not been adequately studied. For example, other critical neurological endpoints associated with repeated inhalation exposure of adult animals to xylenes have been studied only in male rats (Pryor et al., 1987; Korsak et al., 1992, 1994; Gralewicz et al., 1995; Gralewicz and Wiaderna, 2001), and acute controlled exposure studies with human subjects have involved only men (e.g., Gamberale et al., 1978; Savolainen and Linnavuo, 1979; Rhiihimaki and Savolainen, 1980; Savolainen et al., 1984; Olson et al., 1985; Dudek et al., 1990; Laine et al., 1993).

As discussed in Sections 4.5.1 and 4.5.2, available studies of the developmental toxicity of xylenes do not suggest that the developing organism is more sensitive than the adult to xylene exposure. Similarly, no studies are available that have examined whether children may be more susceptible than adults to the effects of xylenes exposure.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

No studies of the toxicity of xylenes in humans following subchronic or chronic oral exposure are available. Available animal studies do not identify a single sensitive health effect other than changes in body weight, which have been seen consistently in male rats across several studies. Decreased body weights (5–8% decrease relative to controls) were observed in male but not female rats exposed to 500 mg/kg-day of mixed xylenes by gavage for 2 years (NTP, 1986). Subchronic studies have also reported decreased body weights: an 11% decrease (compared with controls) in male but not female rats exposed to 1000 mg/kg-day of mixed xylenes for 13 weeks (NTP, 1986); a 6% decrease in male but not female rats exposed to 1500 mg/kg-day of mixed xylenes for 90 days (Condie et al. 1988); a 15% decrease in male rats exposed to 800 mg/kg-day m-xylene for 90 days (Wolfe 1988a); and a 7% decrease in mice exposed to 2000 mg/kg-day mixed xylenes for 13 weeks (NTP, 1986). No changes in body weight were reported in mice exposed for 2 years to mixed xylenes at doses as high as 1000 mg/kg-day (NTP, 1986).

Mortality was dose-related and statistically significantly increased (p=0.04) in high-dose male rats following chronic exposure to mixed xylenes in the NTP (1986) study. Total survival rates (treatment- and gavage-related) were vehicle-control, 36/50; 250 mg/kg-day, 26/50; and 500 mg/kg-day, 20/50. A number of deaths were reported as gavage related. Treatment-related mortality rates were vehicle-control, 11/50; 250 mg/kg-day, 17/50; and 500 mg/kg-day, 19/50. The authors noted that behavioral effects were not recorded during gavage. Survival rates of female rats and both sexes of mice were not statistically significantly different from those of controls; no treatment-related trends were evident.

Increased mortality was also observed in rats in two subchronic studies following exposure to m-xylene (Wolfe et al., 1988a) and p-xylene (Wolfe et al., 1988b). In the first study, statistically significantly increased mortality was observed in the mid-dose (200 mg/kg-day) male and mid- and high-dose (800 mg/kg-day) female rats. In the second study, mortality in high-dose male rats (800 mg/kg-day) attained statistical significance, and a statistically significant trend was present in the male dose groups. In both studies, early mortality may have resulted from treatment-related aspiration of the test material, as evidenced by lung congestion.

Other effects of oral exposure to xylenes appear to occur at greater exposure levels than those associated with decreased body weight and increased mortality. Adverse kidney effects were noted at 750 mg/kg-day but not at 150 mg/kg-day in the 90-day study by Condie et al. (1988). Kidney effects were not found in the NTP (1986) bioassay with Fisher 344/N rats or B6C3F1 mice exposed to xylenes for 13 weeks or 2 years. Likewise, no nephropathy was reported in a nephrotoxicity screening assay in male Fischer 344/N rats exposed to 2000 mg/kg m-xylene for 5 days per week for 4 weeks (Borriston Laboratories, Inc., 1983). In addition, no kidney effects were found in Sprague-Dawley rats exposed for 90 days to m-xylene or p-xylene at doses as high as 800 mg/kg-day (Wolfe et al., 1988a, b). Thus, the available data do not consistently identify the kidney as a sensitive target of xylenes in animals. Likewise, the available data do not consistently identify the liver as a sensitive target of xylenes in animals (NTP, 1986; Wolfe et al., 1988a, b; Condie et al., 1988).

The only neurobehavioral effects noted in any of the available studies occurred in the 2-year NTP (1986) study in mice, which noted hyperactivity immediately following gavage in animals exposed to 1000 mg/kg-day but not 500 mg/kg-day. Developmental effects (decreased fetal body weight and an increase in the incidence of cleft palate) occurred at exposure levels of ~2000 mg/kg-day (Marks et al., 1982; Nawrot and Staples, 1980). An adequate examination of the effects of oral xylenes exposure on reproductive endpoints has not been reported.

The 2-year study in rats conducted by NTP (1986) was selected as the principal study for the derivation of the RfD for xylenes because it is the only oral animal study of chronic duration and because some effects (decreased body weight and possible increased mortality) were evident at doses lower than those that produced effects seen in other studies. The body weight decrease (5–8% of controls') is considered to be of marginal biological significance, but there was a statistically significant trend for decreased survival in male rats with increasing exposure levels, and survival in the high-dose males was statistically significantly decreased when compared with controls. Given the possibility of treatment-related frank toxicity, it is not considered prudent to discount the only other observed effect, that is, decreased body weight. Thus, the highest dose in the study, 500 mg/kg-day, is considered a LOAEL for changes in body weight and mortality.

In the study selected for the principal study, animals were exposed to mixed xylenes. As discussed in Section 4.4.3., although some differences in toxicity were apparent among the individual isomers, there is no consistent evidence for quantitative differences in the potency of the isomers following oral or inhalation exposure.

The selection of the principal study is the same as reported in the previous IRIS assessment. The co-critical effects are the same with the exception of hyperactivity in mice at the high dose of 1000 mg/kg-day. This effect has not been included as a critical effect in the current assessment due to the higher dose required to achieve the effect.

5.1.2. Methods of Analysis

The data were analyzed using the NOAEL/LOAEL approach. Because neither individual body weight data nor group means and standard deviations for body weights were reported in the NTP (1986) report, a benchmark dose (BMD) approach was not feasible. However, a BMD analysis was applied to the body weight effects seen in the subchronic study by Wolfe (1988a) for comparison purposes, resulting in an RfD similar to one derived from the NOAEL/LOAEL approach using the NTP (1986) chronic rat data (see Appendix C). The NOAEL/LOAEL approach using the chronic data and the comparison BMD analysis using the subchronic data are described in Section 5.1.3.

5.1.3. Oral Reference Dose Derivation

The NTP (1986) study identified a NOAEL of 250 mg/kg-day for decreased body weight in male rats exposed by gavage 5 days per week for 2 years. The dose was duration adjusted as follows:

$$\begin{aligned} NOAEL_{[ADJ]} &= \text{NOAEL x 5 days/7days} \\ &= \text{250 mg/kg-day x 5/7} \\ &= \text{179 mg/kg-day} \end{aligned}$$

To the duration-adjusted NOAEL of 179 mg/kg-day, a total uncertainty factor (UF) of 1000 was applied to derive the RfD:

$$\begin{aligned} RfD &= NOAEL_{[ADJ]} \div (UF \times MF) \\ &= \text{179 mg/kg-day} \div (1000 \times 1) \\ &= \text{0.2 mg/kg-day} \end{aligned}$$

The total UF of 1000 (10 x 10 x 10) was derived by applying a UF of 10 to account for laboratory animal-to-human interspecies differences. No information is available to support a change from default.

A UF of 10 was applied for intraspecies uncertainty to account for human variability and sensitive populations. This factor accounts for humans who may be more sensitive than the general population to exposure to xylenes.

A UF of 10 was used to account for data base uncertainty. The available oral data base for xylenes includes chronic and subchronic gavage toxicity studies in mice and rats and a developmental toxicity study. None of these studies indicate that additional data would result in a lower RfD. However, the data base lacks adequate studies of the oral neurotoxicity of xylenes as well as multigenerational reproductive toxicity and developmental neurotoxicity studies.

Given the identification of neurological impairment as a critical health hazard from inhalation exposure to xylenes, the lack of comprehensive neurotoxicity testing following chronic oral exposure is of particular concern. It should be noted that transient neurotoxic effects (e.g., lethargy, tremors and unsteadiness) were reported in mice following oral exposure to xylenes for

13 weeks (NTP, 1986). There are no toxicokinetic data identifying oral dose levels at which first-pass hepatic metabolism of xylenes becomes saturated in animals or humans; such data could decrease uncertainty regarding whether or not neurological impairment may occur at dose levels below those causing body weight decreases and mortality in rats. It is uncertain whether the availability of comprehensive oral neurotoxicity data would result in a lower RfD.

An additional uncertainty associated with the oral data base is that the majority of studies examined mixed xylenes, which are known to contain ethylbenzene. The present IRIS assessment for ethylbenzene (U.S. EPA, 2002), which was entered on the data base in 1987, cites effects on the liver and kidney as the most sensitive endpoints following oral exposure. As discussed above, effects on the liver and kidney have been reported following oral exposure to mixed xylenes, but the most sensitive effect reported in animal bioassays is decreased body weight and increased mortality, as identified by the principal study (NTP, 1986). However, because the mechanism behind the critical effect has not been clearly elucidated, a possible contribution of ethylbenzene to the toxicity of mixed xylenes cannot be entirely eliminated. Additional studies comparing the toxicity of mixed xylenes with that of the individual isomers would better inform the data base.

The RfD is based on a NOAEL from a chronic study, which obviates the need for a UF due to LOAEL to NOAEL extrapolation or subchronic extrapolation.

An RfD was derived for comparison purposes by applying the BMD methodology to the data from the subchronic rat study of Wolfe et al. (1988a) (see Appendix C). Modeling the body weight data with a linear model for continuous data results in a BMDL (lower bound of the benchmark dose) of 440 mg/kg-day, using a 10% change as the benchmark response. Derivation of an RfD from this point of departure using the same UFs as above but with an additional UF of 3 for extrapolation from subchronic to chronic duration (total UF of 3000) would result in a value of 0.1 mg/kg-day, which is similar to the proposed RfD.

The RfD generated in this assessment differs from the previous RfD (2 mg/kg-day). The difference is accounted for by the addition of a data base UF, which was not considered in the previous assessment.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

As discussed in Section 4.5.2., the weight of evidence from limited human data and more extensive animal data identify mild neurological impairment as a sensitive potential health hazard from repeated inhalation exposure to xylenes in the concentration range of 100 to 200 ppm. Evidence for biologically significant developmental toxic effects comes from studies of animals exposed to higher concentrations. Toxicity studies in animals (e.g., Carpenter et al., 1975; Jenkins et al., 1970) have not found consistent evidence for other effects such as changes in body weight or hepatic, hematologic, or renal toxicity endpoints following exposure to

concentrations as high as 800–1000 ppm for 6 hours per day, 5 days per week. In addition, no effects on reproductive performance or histology of reproductive organs were found in CD rats exposed to 500 ppm mixed xylenes for 6 hours per day, 5 days per week, prior to mating and continuing through the end of lactation (Bio/dynamics Inc., 1983).

The discussion of the available data (predominately from animal studies) in Section 4.5.2 illustrates that the weight of evidence for mild neurological impairments following repeated inhalation exposure to xylene concentrations below 200 ppm is greater than the weight of evidence for developmental effects.

Because available human data are insufficient for derivation of an RfC and chronic animal inhalation data are lacking, the subchronic study by Korsak et al. (1994) was selected as the principal study. Neurological effects (impaired motor coordination) were selected as the critical effect for derivation of the RfC. Two neurological endpoints were evaluated in Korsak et al. (1994). Rotarod performance was statistically significantly decreased (33% from controls') at 100 ppm, whereas a statistically significant decreased sensitivity to pain was observed at 50 and 100 ppm (8.6 and 8.7 seconds, respectively, vs. 12.2 seconds for controls; measurements were made 24 hours postexposure). Gralewicz and Wiaderna (2001) also measured the effect of m-xylene exposure (6 hours per day, 5 days per week, for 4 weeks; neurological endpoints measured on post-exposure day 50) on pain sensitivity. In this study, a statistically significant increase in pain sensitivity (35 seconds vs. 10 seconds in control) was found at the 100 ppm dose, the lowest dose tested. The variation in the response to m-xylene in these two studies decreases the confidence in using the pain sensitivity endpoint as the critical effect.

A number of statistically significant neurological effects have been noted in male rats at a dose of 100 ppm m-xylene in other supporting studies: decreased rotarod performance and spontaneous movement activity following exposure for 6 hours per day, 5 days per week, for 6 months (Korsak et al., 1992); decreased radial maze performance following exposure for 6 hours per day, 5 days per week for 3 months (Gralewicz et al., 1995); and shortened step-down time in the passive avoidance test following exposure for 6 hours per day, 5 days per week, for 4 weeks. All the studies measured neurological endpoints 24 hours postexposure, with the exception of Gralewicz and Wiaderna (2001), which measured effects at postexposure day 50. A NOAEL of 50 ppm and a LOAEL of 100 ppm are identified for neurological effects (impaired motor coordination).

The principal study (Korsak et al., 1994) reported no statistically significant exposure-related changes in body weight gain, absolute or relative organ weights, hepatic activities of monoxygenases or lipid peroxidation, or levels of triglycerides in the liver. Compared with controls, exposed rats showed statistically significant changes in red blood cell counts (7–20% decrease), hemoglobin levels (~8% decrease), and white blood cell counts (35% increase). Effects in red blood cell counts and hemoglobin levels were observed at 50 ppm; however, these changes were not observed in another study from the same laboratory in rats exposed to 1000 ppm m-xylene (Korsak et al., 1992). Furthermore, effects on erythrocytes were not found at concentrations of 78–810 ppm in other studies (Carpenter et al., 1975; Jenkins, 1970).

There is some uncertainty associated with selecting a principal study for xylenes that involved exposure to m-xylene alone, but m-xylene is generally the predominant isomer in commercial mixtures. In addition, although there are no studies comparing the effects of xylene isomers on critical neurological endpoints following subchronic or chronic inhalation exposure, the potencies of individual xylene isomers were similar in affecting neurobehavior, as shown in a study of rats following acute exposures (Moser et al., 1985).

5.2.2. Methods of Analysis

A NOAEL/LOAEL approach was used to derive the RfC. A benchmark concentration (BMC) was not derived because the principal study reported the neurobehavioral data only as group means; standard deviations or standard errors for groups or for the individual animal data were not presented. In the absence of numerical and statistical information, BMC modeling cannot be performed. Validated PBPK models for xylene inhalation for rats and humans have been developed (Haddad et al., 1999; Tardif et al., 1991, 1992, 1993a, b, 1995) and were used for comparison purposes in the calculation of a $NOAEL_{[HEC/PK]}$. The results of the calculations are presented in Section 5.2.3.2 and Appendix B.

5.2.3. Inhalation Reference Concentration Derivation

5.2.3.1. Principal RfC Derivation

The NOAEL of 50 ppm (217 mg/m^3) was duration adjusted as follows:
The $NOAEL_{[ADJ]}$ was used to derive a human equivalent concentration (HEC), as

$$NOAEL_{[ADJ]} = NOAEL \times \frac{5\ days}{7\ days} \times \frac{6\ hrs/day}{24\ hrs/day}$$

$$= 217\ mg/m^3 \times \frac{5}{7} \times \frac{6}{24}$$

$$= 39\ mg/m^3$$

described in U.S. EPA (1994b). Xylene is considered a category 3 gas because of its low water solubility and its potential for accumulation in blood during exposure and because its most sensitive effect is an extrarespiratory effect. The $NOAEL_{[HEC]}$ was calculated using the equation

$$\frac{\left(H_{b/g}\right)_A}{\left(H_{b/g}\right)_H} = \frac{46.0}{26.4} = 1.7$$

$$NOAEL_{[HEC]} = NOAEL_{[ADJ]} \times \frac{\left(H_{b/g}\right)_A}{\left(H_{b/g}\right)_H}$$

$$= 39\,mg/m^3 \times 1$$
$$= 39\,mg/m^3$$

$$RfC = NOAEL_{[HEC]} \div (UF \times MF)$$

$$= 39\,mg/m^3 \div (300 \times 1)$$
$$= 0.1\,mg/m^3$$

$$NOAEL_{[HEC]} = NOAEL_{[ADJ]} \times \frac{\left(H_{b/g}\right)_A}{\left(H_{b/g}\right)_H}$$

where $H_{b/g}$ = blood/gas partition coefficient for the species in question, animal (A) or human (H)

Tardif et al. (1995) reported an $(H_{b/g})_H$ of 26.4 for m-xylene, and an earlier study from the same group (Tardif et al., 1993a) reported an $(H_{b/g})_A$ of 46.0 for m-xylene in the rat. It follows that

However, when $(H_{b/g})_A > (H_{b/g})_H$, a value of 1 is used for the ratio (U.S. EPA, 1994b). Therefore,

To the $NOAEL_{[HEC]}$ of 39 mg/m^3, a total UF of 300 was applied to derive the RfC as follows:

The total UF of 300 ($10^{1/2}$ x 10 x $10^{1/2}$ x $10^{1/2}$) was derived by applying a UF of 3 to account for laboratory animal-to-human interspecies differences. A factor of 3 was applied because default $NOAEL_{[HEC]}$ dosimetric adjustments were used to calculate a HEC, reducing the uncertainty involved with the extrapolation from the results of an animal study to a human

exposure scenario (i.e., the toxicokinetic portion of the UF is 1; the toxicodynamic portion is 3). (See Section 5.2.3.2 for further discussion of the toxicokinetic area of uncertainty.)

A UF of 10 was applied for intraspecies uncertainty to account for human variability and sensitive populations. The degree of human variance in abilities to absorb or dispose of xylenes is unknown, as is the degree of human variance in responding to xylenes neurotoxicity. Results from developmental toxicity studies of rats exposed by inhalation during gestation indicate that adverse developmental effects occur only at doses higher than chronic doses producing the critical effects observed in adult male rats in the principal and supporting studies, suggesting that the developing fetus is not at special risk from low-level exposure to xylenes. However, as with oral exposure, the effects of inhaled xylenes in other potentially sensitive populations such as newborns or young children or animals have not been assessed.

A UF of 3 was applied for extrapolation from subchronic to chronic duration. A factor of 10 was not used because the changes in rotarod performance did not increase with time from 1 to 3 months, and they were similar to those described in a separate study of 6 months duration (Korsak et al., 1992).

A UF of 3 was applied for uncertainties in the data base. The inhalation data base includes some human studies, subchronic studies in rats and dogs, neurotoxicity studies, a one-generation reproductive toxicity study, developmental toxicity studies, and developmental neurotoxicity studies. Although the available developmental toxicity studies are confounded by a lack of litter incidence reporting, the data reported for fetal incidences do not indicate effects at levels lower than the level found to induce neurologic impairment in several endpoints in male rats. The data base is lacking a two-generation reproductive toxicity study.

5.2.3.2. PBPK Model Applications

Rat and human PBPK models for xylene inhalation (Tardif et al., 1991, 1992, 1993a, b, 1995; Haddad et al., 1999) were applied to the rat NOAEL of 50 ppm (217 mg/m^3) to explore how the use of these models may influence the derivation of the RfC.

If the $NOAEL_{[ADJ]}$ of 39 mg/m^3 (24 hours per day) is used as the exposure concentration for the rat PBPK model, the model predicts a steady-state pooled venous blood concentration of 0.144 mg/L. This value was then used in the human PBPK model, resulting in an estimated $NOAEL_{[HEC/PK]}$ (continuous inhaled concentration in humans that would result in a steady-state pooled venous blood concentration of 0.144 mg/L) of 41 mg/m^3. This supports the $NOAEL_{[HEC]}$ of 39 mg/m^3 calculated by the standard inhalation dosimetric methods.

Alternatively, the unadjusted NOAEL of 217 mg/m^3 (50 ppm) and the actual exposure protocol used in the Korsak et al. (1994) rat study (6 hours per day, 5 days per week, for 3 months) were used in the rat PBPK model to predict arterial blood concentration in rats as a function of time up to 13 weeks. The results show a daily rise and fall of xylene concentrations,

consistent with rapid elimination from the blood (see Appendix B, Figure 4). The use of three different dose surrogates was explored in extrapolating to HECs with the human PBPK model:

- an overall TWA blood concentration (0.198 mg/L, averaged over 1-hour intervals across 13 weeks),
- the maximum (MAX) blood concentration attained on any given day during exposure (1.09 mg/L, essentially a constant over 13 weeks), and
- the mid-point (MID) concentration between the maximum (1.09 mg/L) and the minimum (0 mg/L) concentration on any given day during exposure (0.55 mg/L).

Using these values as potential dose surrogates in the human model, the model predicted air concentrations that would produce these steady-state concentrations in human blood with continuous exposure. As shown in Figures 5, 6, and 7 in Appendix B, air concentrations predicted to attain these steady-state blood concentrations in humans with continuous exposure were 10.5 ppm (46 mg/m^3) for the TWA surrogate, 27.4 ppm (106 mg/m^3) for the MID, and 49.8 ppm (216 mg/m^3) for the MAX.

The rat model predicts that blood concentrations would be essentially zero when the critical effects on rotarod performance were measured (24 hours after cessation of exposure). This supports the idea that the observed effects are not dependent on the concurrent presence of xylenes in the blood and that they may be persistent neurological effects. A better dose surrogate for use in the model would be brain concentrations, but the model has not been developed in that regard.

The TWA dose surrogate is likely to provide a more accurate description of exposure to the rats in the study than the MID or MAX dose surrogates, especially since the effects were measured after m-xylene had completely cleared from the blood. Thus, using the TWA as the dose surrogate in extrapolating from the rats in the Korsak et al. (1994) study to humans, the model predicts a HEC of 46.5 mg/m^3. This is very similar, although not identical, to the HEC concentration (39 mg/m^3) predicted using the default $NOAEL_{[HEC]}$ dosimetry methodology and provides support for the RfC derived in Section 5.2.3.1.

5.3 CANCER ASSESSMENT

Human epidemiological studies have found statistically increased incidence of cancer, but these studies are limited by the number of subjects in the cohort and the low number of incidence reported, and the results are confounded by exposures to other solvents. The animal carcinogenicity data base for xylenes is limited to an NTP (1986) oral bioassay in rats and mice and an oral bioassay in rats reported by Maltoni et al. (1983, 1985). NTP (1986) found *no evidence* of carcinogenicity of xylenes in rats or mice of either sex. In contrast, Maltoni et al. (1983, 1985) reported an increase in total malignant tumors in female but not male rats following exposure to xylenes. However, the increased incidence was calculated by combining different

types of tumors across tissue sites. Incomplete reporting of site-specific tumor incidence data and pathology by Maltoni et al. limit the usefulness of this bioassay in evaluating the carcinogenicity of xylenes. Results from genotoxicity studies have been consistently negative.

Under the *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999) *data are inadequate for an assessment of human carcinogenic potential* of xylenes due to inadequate evidence of carcinogenicity in humans and animals. Because the available human and animal studies provide inadequate evidence of carcinogenicity, no estimates of dose-response relationships can be made.

5.3.1. Oral Exposure

Not applicable.

5.3.2. Inhalation Exposure

Not applicable.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE-RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Xylenes (CASRN 1330-20-7) have the chemical formula C_8H_{10} [structural formula $(CH_3)_2C_6H_4$] and a molecular weight of 106.17. Although liquid at room temperature, xylenes have a low vapor pressure, resulting in extensive volatilization into the air. Commercial or mixed xylenes are comprised of three isomers: *meta*-xylene (m-xylene), *ortho*-xylene (o-xylene), and *para*-xylene (p-xylene), of which the m-isomer usually predominates (44–70% of the mixture). The exact composition of the isomers is commonly dependent on the source. Mixed xylenes are used in the production of the individual isomers or ethylbenzene, as a solvent, in paints and coatings, or as a blend in gasoline.

Data on the effects of xylenes in humans following oral exposure are not available. Results from several subchronic studies in rats and mice and one chronic study in male rats identify decreased body weight and increased mortality as potential health hazards from repeated oral exposure to doses generally greater than 500–800 mg/kg-day. Studies of orally exposed animals provide some evidence for other noncancer effects at a few sites, but these appear to occur either at doses above the lowest levels inducing body weight changes or inconsistently across studies. Results from two animal studies indicate that developmental effects (e.g., cleft palate) are a potential hazard from oral exposure to xylenes but that they occur at doses greater than those inducing body weight changes.

Reversible symptoms of neurological impairment and irritation of the eyes and throat are well-known health hazards from acute inhalation exposure to xylenes and other aromatic solvents in humans. In general, these acute effects are most pronounced at high exposure levels, in excess of 1000 ppm; at lower concentrations, more subtle effects may occur. Animal studies more clearly identify neurological effects as sensitive effects of repeated inhalation exposure to xylenes. Several studies involving subchronic inhalation exposure of rats to m-xylene identified 100 ppm for 6 hours per day as an exposure level that produced statistically significant changes in several neurologic endpoints, including impaired rotarod performance and decreased motor activity, which are indicative of motor coordination; altered radial maze performance, which is indicative of possible spatial learning impairment; and increased sensitivity to pain. Tests of these endpoints were conducted at least 24 hours after exposure ceased, thus providing some evidence that the changes were persistent.

A number of studies have examined the potential developmental effects of airborne mixed xylenes or xylene isomers in animals, but adverse effects have been reported only at exposure levels greater than those at which neurological effects have been reported.

6.2. DOSE-RESPONSE

6.2.1. Noncancer/Oral

No studies of the toxicity of xylenes in humans following subchronic or chronic oral exposure are available. No thorough chronic studies of the noncancer toxicity of xylene in animals are available in the literature. Noncarcinogenic endpoints reported for a lifetime oral carcinogenicity bioassay in rats (Maltoni et al., 1983, 1985) were restricted to body weight and hematologic variables, which were reported to have been without effect in rats exposed to 500 mg/kg-day xylenes. In contrast, in the NTP (1986) 2-year carcinogenicity bioassay in rats and mice, male but not female rats exposed by gavage to 500 mg/kg-day for 5 days per week showed a statistically significant increase in mortality and a statistically significant decrease (5–8%) in mean body weight.

Subchronic studies have also reported decreased body weights: an 11% decrease (compared with controls) in male but not female rats exposed to 1000 mg/kg-day of mixed xylenes for 13 weeks (NTP, 1986), a 6% decrease in male but not female rats exposed to 1500 mg/kg-day of mixed xylenes for 90 days (Condie et al. 1988), a 15% decrease in male rats exposed to 800 mg/kg-day m-xylene for 90 days (Wolfe 1988a), and a 7% decrease in mice exposed to 2000 mg/kg-day mixed xylenes for 13 weeks (NTP, 1986). No changes in body weight were reported in mice exposed for 2 years to mixed xylenes at doses as high as 1000 mg/kg-day (NTP, 1986).

Changes in body weight and increased mortality in rats in the chronic NTP (1986) bioassay were selected as the critical effect for derivation of an RfD. The NOAEL of 250 mg/kg-day was duration adjusted to 179 mg/kg-day and divided by a total UF of 1000 (10 x 10 x 10) (10 for animal-to-human extrapolation, 10 for intrahuman variability, and 10 for deficiencies

in the data base, including a lack of studies examining reproductive and neurotoxic effects) to derive the RfD of 0.2 mg/kg-day.

6.2.2. Noncancer/Inhalation

Although adequate subchronic or chronic data in humans exposed to xylene by inhalation are lacking, acute controlled-exposure studies have identified self-reported symptoms of irritation (e.g., watering eyes and sore throat) or neurological impairment (e.g., mild nausea, headache, altered reaction time, altered balance) as potential effects of xylene following inhalation exposure in humans. However, results from subchronic animal studies identify neurological impairment and possible developmental effects as potential health hazards from repeated inhalation exposure. Scattered reports of body weight changes and adaptive liver changes in animals are available, but the results do not consistently identify these effects as potential health hazards. Several studies have examined the potential developmental toxicity of mixed xylenes and xylene isomers, but they have identified adverse effects only at levels considerably greater than those at which neurological effects have been reported.

The subchronic rat study by Korsak et al. (1994) was selected as the principal study for derivation of the RfC. A NOAEL of 50 ppm and a LOAEL of 100 ppm were identified for decreased rotarod performance (impaired motor coordination). This neurologic test was administered 24 hours after termination of the exposure period, when xylenes would be expected to have been eliminated from the body. Other subchronic rat studies provide support for the finding that 100 ppm exposure produces statistically significant changes in a number of neurological endpoints: decreased rotarod performance (Korsak et al., 1992), decreased spontaneous motor activity (Korsak et al., 1992), and impaired radial maze performance indicative of a learning deficit (Gralewicz et al., 1995).

Additional support for 100 ppm as an exposure level that may produce mild neurologic deficits comes from the report (Gralewicz and Wiaderna, 2001) that rats exposed to 100 ppm m-xylene for 4 weeks showed shortened step-down time in 1/6 trials in the passive avoidance test 50 days postexposure. These studies collectively identify 100 ppm as the lowest reliable subchronic animal LOAEL and 50 ppm as a NOAEL for deficits in neurologic endpoints.

The NOAEL of 50 ppm (217 mg/m^3) was duration adjusted to 39 mg/m^3, and a $NOAEL_{[HEC]}$ of 39 mg/m^3 was calculated on the basis of species differences in blood/gas partition coefficients. The $NOAEL_{[HEC]}$ of 39 mg/m^3 was divided by a total UF of 300 ($10^{1/2}$ x 10 x $10^{1/2}$ x $10^{1/2}$; 3 for animal-to-human extrapolation using default dosimetric adjustments, 10 for intrahuman variability, 3 for extrapolation from subchronic to chronic duration, and 3 for deficiencies in the data base, including lack of studies in two species [available studies are predominantly in male rats] and a two-generation reproductive toxicity study) to give the RfC of 0.1 mg/m^3. Alternative approaches using available PBPK models to extrapolate rat exposure concentrations to HECs arrived at similar points of departure for the RfC as the $NOAEL_{[HEC]}$ of 39 mg/m^3.

6.2.3. Cancer/Oral and Inhalation

Data in both humans and animals are inadequate to evaluate potential associations between xylene exposure and cancer. A number of human occupational studies have suggested possible carcinogenic effects of chronic inhalation exposure to xylene. However, in each case co-exposure to other chemicals was a major confounding factor, leading to an inability to adequately assess the potential effects of chronic exposure to xylene. Animal data on the carcinogenicity of xylene following inhalation exposure are not available. Human data on the carcinogenic effects of oral exposure to xylenes are not available, and animal data provide inadequate evidence of the carcinogenicity of xylenes. Under the *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999) *data are inadequate for an assessment of the carcinogenic potential* of xylenes.

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APPENDIX A. EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for xylenes have undergone both internal peer review by scientists within EPA and a more formal external review by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1994c). Comments made by the internal reviewers were addressed prior to submitting the documents for external review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows. EPA also received scientific comments from the public. These comments and EPA's response are included in a separate section.

Scientific Comments from the External Peer Review

(1) General Comments

A. Editorial/grammatical suggestions

Comment: All three reviewers made editorial comments on the documents. A number of comments related to a lack of sufficient detail in study descriptions.

Response: Where possible, the suggested editorial changes have been implemented. Study descriptions have been augmented throughout the document.

B. Rationale for health assessments

Comment: All three reviewers commented that the rationale for selection of principal studies and critical effects as well as the weight of evidence sections were inadequate.

Response: The appropriate sections have been revised in accordance with the reviewers' comments to clarify the selection of principal studies and critical effects and to more clearly describe the weight of evidence for noncancer health effects from oral and inhalation exposure.

C. Lack of concordance between oral and inhalation reference values

Comment: One reviewer commented extensively on the lack of a direct concordance between the RfD and the RfC, noting that the RfC may be too low. In order to check on the appropriateness of the RfC, the reviewer calculated a daily dose, based on the RfC of 0.1 mg/m^3, of 0.017 mg/kg-day for a 70 kg human, assuming a 60% absorption, and compared that with the RfD proposed in the external peer review draft of 0.7 mg/kg-day. The reviewer noted that ratio of the RfD to the estimated average daily absorbed dose delivered by the RfC (40:1) indicated

that xylenes may be 40 times more toxic by inhalation than by oral administration and expressed the opinion that this is likely to be "grossly incorrect." The reviewer noted that the apparent discrepancy could be due to the duration adjustment that was made in calculating the $NOAEL_{[HEC]}$ of 39 mg/m^3 from the rat experimental concentration of 217 mg/m^3, 6 hours per day, 5 days per week, pointing out that such an adjustment would not be justified if the neurobehavioral effects noted in the principal rat study were of an acute rather than a persistent nature. The reviewer suggested that PBPK analysis may help to rectify this apparent discrepancy but noted that this difference should be either justified or corrected.

Response: Several changes were made in the document in response to the reviewer's comments. Text describing and evaluating the rat and human PBPK models for xylenes is included in Section 3.5 and Appendix B. The new text notes that existing models are for inhalation and do not have an oral portal of entry. As such, they are not useful for route-to-route extrapolation. Application of the existing models to the derivation of the RfC was explored, and the results of this analysis are presented in Appendix B and in Sections 5 and 6. The analysis indicated that the $NOAEL_{[HEC]}$ derived with the default inhalation dosimetry methodology for category 3 gases was similar to the HEC predicted by the PBPK models, providing support for the RfC.

In addition, the new text specifies that the neurobehavioral tests in the principal and supporting studies for the RfC appeared to have been administered 24 hours after exposure ceased, providing support that the observed effects may be persistent rather than acute effects that are dependent on the presence of xylenes in the blood. The revised document notes that blood concentrations were used as dose surrogates in applying the models and that brain concentrations would be a better dose surrogate. However, models have not been developed to predict brain concentrations of xylenes. Other changes were made (principally in Section 3.3) to more completely discuss what is known concerning the first-pass metabolic effect associated with oral exposure to xylenes.

In relation to the potential discordance between the RfD and RfC identified by the reviewer, it may be more appropriate to compare the two points of departure (POD) using physiological data for the rat. Using the reviewers' simple calculation, this would involve multiplying the duration adjusted inhalation POD (39 mg/m^3) times the rat minute volume (0.33 m^3/day) times the presumed absorption of 0.6 (from human data), and then dividing by the rat body weight of 0.180 kg. This yields an estimate of systemic intake of 42.9 mg/kg-day, which is only 1/4 the oral POD (200 mg/kg-day) chosen for the RfD. Whether fortuitous or having scientific basis, the implication is that of concordance of the oral and inhalation data, particularly given the knowledge that first pass metabolism by the oral route occurs.

Furthermore, the RfD on which the reviewer based his calculations has been replaced with a new RfD, which was derived in response to external peer review comments. The principal study, critical effect, and point of departure for the derivation of the RfD have changed as noted. The updated value of the RfD is 0.2 mg/kg-day versus the value of 0.7 mg/kg-day proposed in the external peer review draft. The ratio of the current RfD and the reviewer's

estimated daily absorbed dose associated with exposure to the RfC is now 11 rather than the previous value of 40.

D. Reproductive and developmental data

Comment: One reviewer recommended additional consideration and descriptions of developmental/reproductive toxicity studies. This reviewer indicated that the weight of evidence shows consistent developmental effects across studies.

Response: The reproductive and developmental data have been re-evaluated, and expanded study descriptions are included in Section 4.3. The text in Section 4.5.2. includes a more complete rationale and explanation of the weight of evidence indicating that neurobehavioral effects are a more sensitive health effect than are effects on the developing organism following inhalation exposure to xylene.

E. Other comments

Comment: One reviewer recommended the inclusion of tables summarizing the NOAELs/LOAELs for the oral and inhalation studies.

Response: Two tables, one summarizing the available neurobehavioral studies in adult male rats and one summarizing the pertinent developmental studies (and their NOAELs and LOAELs), have been added to Section 4.5.2, outlining the weight of evidence for the inhalation data. The oral data base was not as extensive as the inhalation data base and was therefore discussed in paragraph form.

Comment: One reviewer noted that the contribution of ethylbenzene to the toxicity of mixed xylenes should be discussed.

Response: A discussion of the possible role of ethylbenzene in the oral effects of mixed xylenes has been added to Section 5.1.3, in the discussion of uncertainties in the data base. Additional discussion of the potential contribution of ethylbenzene to the oral effects of xylenes is included in Sections 4.5.1. and 4.4.3. An equivalent section was not added to Section 5.2.3 because the inhalation RfC is not based on a study that used mixed xylenes. The critical effects for the current inhalation RfC for ethylbenzene are mild developmental effects and skeletal variants observed in rats exposed to 1000 ppm ethylbenzene (U.S. EPA, 2002). Although developmental effects are an endpoint of concern for inhalation of xylenes, the available data suggest that neurological effects are a more sensitive endpoint.

Comment: Two reviewers suggested that a summary paragraph be added to the toxicokinetic section of the document.

Response: A brief summary is included at the beginning of Section 3.

(2) Specific Charge Questions

Question 1. Are you aware of any other data/studies that are relevant (i.e., useful for hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Comments: External reviewers identified a number of studies that were not cited, including a male rat reproductive toxicity study (Nylén et al., 1989), several toxicokinetic studies in animals (Turkall et al., 1992; Kaneko et al., 1993, 1995), several case-control studies of spontaneous abortions in solvent-exposed female workers (Taskinen et al., 1989), and several PBPK modeling studies (Tardif et al., 1991, 1992, 1993a, b; Haddad et al., 1999).

Response to Comments: In response to the reviewers' comments, discussions of these studies were incorporated into the document. A review and evaluation of the male rat reproductive toxicity study by Nylén et al. (1989) are included in Section 4.3.1.2. Descriptions and evaluations of available rat and human PBPK models for inhaled xylene as well as discussion of their application to the derivation of the RfC are now included in Sections 3.5, 5.2.2., 5.2.3, and Appendix B. Toxicokinetic data published by Turkall et al. (1992) and Kaneko et al. (1993, 1995) are discussed in detail in Sections 3.1. and 3.5. Case-control studies by Taskinen et al. (1989, 1994) are evaluated in Section 4.1.2.1.

Question 2. For the RfD and RfC, has the most appropriate critical effect been chosen? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and whether the effect and the species in which it is observed is a valid model for humans.

Comments: For the RfD, all three reviewers questioned the choice of hyperactivity seen at 2000 mg/kg-day in the 13-week NTP mouse study as the critical effect, particularly given (a) the availability of a chronic study, (b) the presence of mortality at 500 mg/kg-day in the chronic rat bioassay, and (c) the apparent acute nature of the effect (persisted only a short time after exposure each day). All three reviewers acknowledged the difficulty in selecting an appropriate endpoint for deriving an RfD. Alternate endpoints that may be appropriate for deriving an RfD were suggested: the developmental effects (cleft palate and reduced fetal body weight) reported by Marks et al. (1982) or the reduced body weight found in the NTP (1986) chronic and subchronic studies. All reviewers discussed the concern that decreased body weight should be used as a critical effect unless a stronger case could be made to discount this effect.

One reviewer indicated that increased mortality should at least be considered in the dose-response evaluation but that this effect should not be the critical effect. Another reviewer suggested using the LOAEL for hypersensitivity in mice from the chronic study (NTP, 1986) over the LOAEL for subchronic clinical effects (NTP, 1986) in deriving the RfD. This reviewer stated that using the subchronic study over the chronic study had too many shortcomings. In addition to being of shorter duration, the subchronic study only used 10 animals/sex/group, whereas the chronic study used 50 animals/sex/group. In addition, it was pointed out that

comprehensive histopathology was conducted on all animals in the chronic study but only in the vehicle control and high-dose animals in the subchronic study.

For the RfC, all three reviewers agreed that the selection of the critical effect was appropriate. However, it was pointed out that another study (Gralewicz et al., 1995) identified effects at the same dose (i.e., 100 ppm) but was not included as a co-principal study. One reviewer thought that there was also some evidence of human effects at lower doses (14 ppm), as identified in the Uchida et al. (1993) study, that should be addressed. Another reviewer thought that hematological effects were discounted without sufficient explanation.

Response to Comments: As per the reviewers' comments, the critical effect for the RfD has been re-evaluated and changed to reduced body weight and increased mortality, based on the evidence from subchronic and chronic rat studies following oral xylenes exposure. A new RfD has been derived using decreased body weight and mortality as the critical effect. The developmental NOAEL and LOAEL identified by Marks et al. (1982) were not used because the available LOAEL values for decreased body weight were lower, indicating a more sensitive effect. Sections 4.5.1 and 5.1 have been extensively modified, reflecting the new principal study and critical effect.

The weight of evidence for the inhalation data and the rationale for the RfC have been updated and enhanced, with additional discussion of the neurotoxicity data, including the Gralewicz et al. (1995) study and the addition of a later study by the same group (Gralewicz and Wiaderna, 2001). The limitations of the Uchida et al. (1993) study are more fully discussed in Section 4.5.2. Hematological effects reported by Korsak et al. (1992, 1994) are more fully described.

Question 3. Have the cancer and noncancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or appropriate precursor) in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

Comments: For the RfD, none of the reviewers concurred with the selection of the 13-week mouse study by NTP (1986) as the principal study. One reviewer suggested that the study of Marks et al. (1982) be selected as the principal study and two others questioned using the subchronic portion of the NTP (1986) study, which identified a higher LOAEL, over the chronic portion of the NTP study.

Regarding the selection of the principal study for deriving the RfC, all three reviewers agreed that the most appropriate principal study had been chosen. All three reviewers agreed that the cancer classification was appropriate and that the data were not adequate for derivation of an oral slope factor or inhalation unit risk, although one suggested that a classification of "no evidence of carcinogenicity in adequately conducted cancer bioassays in two sexes of two species" be used. Two reviewers suggested that the limitations of the Maltoni et al. (1983, 1985) study be more fully discussed.

Response to Comments: As per the reviewers' comments, the oral data base has been re-evaluated, and the NTP (1986) chronic study in rats has been selected as the principal study for derivation of the RfD, using the critical endpoints of decreased body weight and increased mortality in male rats. As discussed above, the study by Marks et al. (1982) was not selected because it identified a considerably higher LOAEL than was identified in NTP (1986). Section 5.1 has been extensively modified, reflecting the change in the principal study.

A more complete discussion of the limitations of the Maltoni et al. (1983, 1985) study is included in Section 4.6, as per the reviewer's suggestion. A classification of "not likely to be carcinogenic in humans" was not adopted because although the Maltoni et al. (1983, 1985) study has several limitations, which were outlined by the reviewer, it was considered to add sufficient doubt to the classification.

Question 4. Studies included in the RfD and RfC sections under the heading "Supporting/Additional studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, and any evidence corroborative of the critical effect or to establish the comprehensiveness of the data base with respect to various endpoints (reproductive/developmental toxicity studies, for example). Should other studies be included under the supporting/additional studies category? Should some studies be removed? Do you agree with the selection of the NOAEL/LOAEL for determining the RfD, given the manner in which the data are reported?

Comments: For the RfD, all three reviewers commented that the selection of the LOAEL of 2000 mg/kg-day from the NTP (1986) subchronic mouse study was not the appropriate selection, mainly due to difficulties with the critical endpoint, as discussed above. One reviewer suggested that additional studies or findings be presented to support the LOAEL, and another suggested the possibility of using Marks et al. (1982) as a co-critical study, as it identified a similar LOAEL. One reviewer noted that insufficient study detail was provided in both the principal and supporting studies section and the additional studies/comments section.

One reviewer commented that the selection of the LOAEL for the RfC seemed to be a reasonable choice; the other two reviewers made no comment as to the appropriateness of the NOAEL and LOAEL selected. No reviewer expressed dissatisfaction with the evidence in support of the RfC, although one suggested that the study descriptions be clarified and NOAEL/LOAEL values be clearly identified where appropriate. One reviewer indicated that Gralewicz et al. (1995) should be included in the IRIS Summary as a supporting study because effects were noted at the same point of departure as that of the critical effect.

Response to Comments: The RfD derivation has been changed. A new RfD has been derived on the basis of decreased body weight and increased mortality in the chronic NTP (1986) study in rats. The majority of the reviewers' comments regarding the RfD have been addressed in the replacement of the NOAEL and LOAEL in question with more appropriate values. Additional detail has been added to study descriptions, including the identification of

NOAEL/LOAEL values. The discussion of the RfC rationale has also been expanded and includes the rationale for selection of critical study and supporting studies.

Question 5. For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors (UFs) or the modifying factor? Do you consider that the data support the use of different (default) values than those proposed?

Comments: Two reviewers suggested that the application of pharmacokinetic models be considered to reduce the uncertainty of extrapolation from animals to humans for the RfD and RfC. One reviewer felt the existing UFs for the RfD were appropriate and one suggested that a threefold UF for data base insufficiency would be appropriate rather than the existing 10 in the external peer review draft because of adequate chronic and subchronic studies in two species and two developmental studies in rats in the oral data base. This reviewer stated that the inhalation data base further supports the identification of the same endpoint (i.e., neurotoxicity). This reviewer also stated that a subchronic-to-chronic extrapolation UF of 3 was warranted. The third reviewer only commented on the interspecies UF as discussed above.

Regarding the UFs for the RfC, one reviewer commented that the existing UFs (3 each for dosimetric adjustments, data base insufficiency, and duration extrapolation and 10 for intraspecies variation) were appropriate. Another reviewer suggested that a threefold UF might not be sufficient for extrapolation from a subchronic-to-chronic study. This reviewer thought that a combined UF of 10 should account for subchronic-to-chronic extrapolation and data base deficiencies.

Response to Comments: As a result of other comments, the previous RfD has been removed and a new value has been derived on the basis of decreased body weight and increased mortality in the chronic NTP (1986) rat study; therefore, the current UFs differ from those in the external peer review draft. The current UFs include a 10 for animal-to-human extrapolation, a 10 for intrahuman variability, and a 10 for deficiencies in the data base, for a total UF of 1000. The UF of 3 for subchronic-to-chronic extrapolation has been removed, as the critical effect is now based on a chronic study. The data base UF remains at 10. Additional information is included in Section 5.1.3.

The UFs for the RfC remain as described because the reviewers were generally in agreement regarding the choice of UFs for the RfC. A discussion of the potential applicability of the available PBPK models is included above. The available models do not include an oral input component, making application to the derivation of the RfD not feasible. Application of the models to extrapolate exposure levels from animal to humans has been performed to support the RfC derivation in response to peer review comments. The PBPK model calculations do not alter the final RfC value. This information is discussed in Sections 3.5, 5.2.2, and 5.2.3 and Appendix B.

Question 6. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects

(cancer and noncancer) to humans, and the comprehensiveness of the data base? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Comments: Two reviewers suggested that the confidence in the RfD be low rather than low-to-medium, whereas the third did not suggest a change. All reviewers agreed that the confidence statement concerning the RfC was appropriate.

Response to Comments: Because a new RfD has been derived, a new statement of confidence has been prepared, reflecting medium confidence in the new RfD. The confidence level of the RfC remains at medium.

Scientific Comments from the Public

The commentor questioned the application of a 10 for the data base UF for the RfD. The commentor stated that there are multiple studies by both the inhalation and oral routes of exposure and that these studies should be sufficient to reduce the data base UF.

EPA Response: The available oral data base for xylenes includes chronic and subchronic gavage toxicity studies in mice and rats and a developmental toxicity study. The chronic studies were conducted largely as cancer bioassays. The data base lacks adequate studies of the oral neurotoxicity of xylenes as well as multigenerational reproductive toxicity and developmental neurotoxicity studies.

Given the identification of neurological impairment as a critical health hazard from inhalation exposure to xylenes, the lack of comprehensive neurotoxicity testing following repeated oral exposure is of particular concern. There are no toxicokinetic data identifying oral dose levels at which first-pass hepatic metabolism of xylenes becomes saturated in animals or humans; such data could decrease uncertainty regarding whether or not neurological impairment may occur at dose levels below those causing body weight decreases and mortality in rats. For these reasons, the data base UF for the RfD remains at 10.

The commentor raised issues about the interpretation of results in the Korsak et al. (1994) study that was used for the derivation of the RfC. Specific issues included the following: (1) the only significant finding for m-xylene from this study is the rotarod performance decrement effect; and (2) there is no mention of whether the rotarod tests were conducted immediately post-exposure or somewhat later. This significant omission makes it much more difficult to judge whether the performance decrements observed are transient or permanent. The commentor also stated that the composite UF applied to the RfC is too high given the "mild" nature of the effects reported in the critical study.

EPA Response: (1) Justification for selection of the critical effect has been augmented in Section 5.2.1. Briefly, additional discussion of supporting studies in which neurological effects have been reported including altered pain sensitivity, motor coordination, and cognitive ability has

been added. Collectively, these data indicate that xylenes are neurotoxic and that decreased rotarod performance is a viable critical effect.

(2) In the Korsak et al. (1992) study measurements were made 24 hours post-exposure: given the similarity between the two studies, a reasonable assumption was made that measurements in the Korsak et al. (1994) study were conducted using the same protocol. This information has been added to the study description and Section 5.2.1. Additionally, Gralewicz and Wiaderna (2001) reported altered pain sensitivity 50 days post exposure indicating a more permanent effect from exposure to xylenes. As noted in Section 5.2.3.2, application of the Tardif models (Tardif et al., 1991, 1992, 1993a, 1993b, 1995; Haddad et al., 1999) for interspecies extrapolation indicates that the blood concentration of xylenes would be essentially zero when responses were observed 24 hours post exposure in the Korsak et al. (1992, 1994) studies also suggesting that effects could be persistent.

The basis for the choice of uncertainty factors has been augmented in light of additional studies that were identified during the external review process. The text in Section 5.2.3.1 has been modified accordingly.

The commentor emphasized that the assessment should incorporate recent developments in pharmacokinetics for the derivation of reference values for xylenes and specifically identified a pharmacokinetic model by Pelekis et al (2001) which could be used to justify a reduction in the intraspecies UF for the RfD and RfC. [Pelekis, M; Gephardt, LA; Lerman, SE. (2001) Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile compounds. Regul. Toxicol. Pharmacol. 33:12-20].

EPA Response: In response to the general comment concerning the application of pharmacokinetic information in the assessment, a PBPK model was considered for the derivation of the RfC. This information is now presented in Section 5.2.3.2. The point of departure derived from the model is essentially the same as that reported from a direct application of the rat data.

In brief, the Pelekis model was developed to apply pharmacokinetic information to derive a chemical-specific intraspecies UF. The result of the effort is an informed quantitation of "normal" human-to-human and adult-to-child variability. The modeling is specific for the inhalation route of exposure; thus, consideration for the oral route was not possible. It should be noted that the model lacks a specific component for the target organ, i.e., the brain.

*The concept of a chemical-specific intraspecies UF for xylenes is intriguing; however, the Pelekis model is based solely on the pharmacokinetic differences between adults and children. While it is common to divide the inter**species UF into pharmacokinetic and pharmacodynamic portions, it is not readily evident that this "simple" apportionment would apply to the intra**species UF. In the case of intraspecies variability, the differences in humans may be due to lifestage (childhood versus advanced age), genetic polymorphisms, decreased renal clearance in disease states, unknown pharmacodynamic variations in response to xylenes exposure, etc. It is*

not clear that the variability defined in the Pelekis model accounts for the differences in pharmacokinetics of these various human states. In addition, it is not clear what additional contributors to intraspecies variability (both pharmacokinetic and pharmacodynamic) would need to be quantitated and combined to derive a chemical-specific intraspecies UF for xylenes. It is likely that a better understanding of the mode of action would be necessary.

APPENDIX B. PBPK MODELS FOR m-XYLENE

B.1. Structures of the models

Both rat (Tardif et al., 1991, 1992, 1993a) and human (Tardif et al., 1993b, 1995; Haddad et al., 1999) PBPK models for m-xylene inhalation have been developed. These models, developed both individually and for use in modeling of mixtures of other solvents (e.g., benzene, toluene, and ethylbenzene), predict blood and tissue concentrations from air concentrations on the basis of partition coefficients, blood flow rates, and ventilation rates.

Conceptually, the models consist of five dynamic tissue compartments, representing the lung, adipose tissue, slowly perfused tissues, richly perfused tissues, and the liver. A visual depiction of the model is provided in Figure B2. Inhalation of xylenes is represented by addition of xylenes to the system via the lung component. Concentration in arterial blood is predicted on the basis of the existing venous blood concentration, the rate of xylenes exhalation, the inhaled xylenes concentration, and the blood/gas partition coefficient. The concentration in each tissue compartment is predicted on the basis of the existing tissue concentration and the arterial concentration, using appropriate tissue/blood coefficients. Metabolism is assumed, for purposes of the model, to occur only in the liver compartment and is described by a series of equations that assume a saturable process characterized by a V_{max} (maximal velocity for metabolism, in mg/hr) and K_m (Michaelis-Menten affinity constant). The pooled venous concentration is calculated as a mean concentration, based on the blood flow rates from each compartment and the concentration of blood leaving each compartment.

Physiological parameters used in the application of the PBPK models are shown in Table B1. For the human models, cardiac output and alveolar ventilation rate were calculated using the equation Q=18 L/hr-kg x (body weight)$^{0.70}$. For the rat models, these parameters were calculated using Q=15 L/hr-kg x (body weight)$^{0.75}$. Other physiological parameters for the models were obtained from the literature (Arms and Travis, 1988; Gargas et al., 1989; Purcell et al., 1990; Kaneko et al., 1991). Validation of the models following inhalation exposure in both rats (Tardif et al., 1993a, 1997) and humans (Tardif et al., 1995, 1997) has been reported. These models have also been applied to mixtures containing xylenes and other aromatic solvents.

B.2. Application of the models to derive human equivalent concentrations (HECs)

If the $NOAEL_{[ADJ]}$ of 39 mg/m^3 is used as the exposure concentration for the rat PBPK model, the model predicts a steady-state pooled venous blood concentration of 0.144 mg/L. This value was used in the human PBPK model, resulting in an estimated $NOAEL_{[HEC/PK]}$ (continuous inhaled concentration in humans that would result in a steady-state pooled venous blood concentration of 0.144 mg/L) of 41 mg/m^3. This supports the $NOAEL_{[HEC]}$ of 39 mg/m^3 calculated by the standard inhalation dosimetry methods.

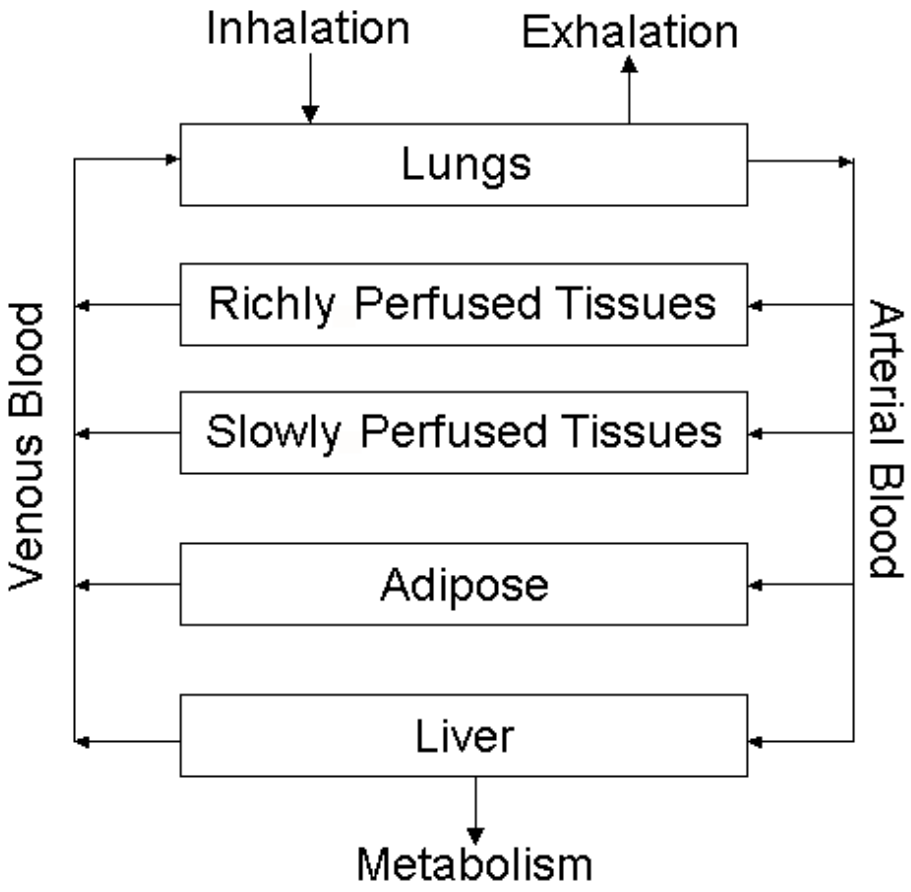


Figure B1. Schematic representation of the PBPK model for xylene.

Alternatively, the unadjusted NOAEL of 217 mg/m^3 (50 ppm) and the actual exposure protocol used in the Korsak et al. (1994) rat study (6 hours per day, 5 days per week, for 3 months) were used in the rat PBPK model to predict arterial blood concentration in the rats as a function of time up to 13 weeks. The results show a daily rise and fall of m-xylene concentrations, consistent with rapid elimination from the blood (Figure B2).

The use of three different dose surrogates was explored in extrapolating to HECs with the human PBPK model:

- an overall time-weighted-average (TWA) blood concentration (0.198 mg/L; averaged over 1-hour intervals across 13 weeks);

Table B1. Physiological parameters and partition coefficients used in the PBPK models

Parameters	Values	
	Rat	Human
Alveolar ventilation rate (L/hr-kg)	15.0	18.0
Cardiac output (L/hr-kg)	15.0	18.0
Fraction of cardiac output corresponding to each compartment		
Fat	0.09	0.05
Slowly perfused tissues	0.15	0.25
Richly perfused tissues	0.51	0.44
Liver	0.25	0.26
Fraction of body weight corresponding to each compartment		
Fat	0.09	0.19
Slowly perfused tissues	0.72	0.62
Richly perfused tissues	0.05	0.05
Liver	0.049	0.026
Partition coefficients		
Blood/air	46.0	26.4
Fat/blood	40.4	77.8
Slowly perfused tissues/blood	0.91	3.0
Richly perfused tissues/blood	1.97	4.42
Liver/blood	1.97	3.02

- the maximum (MAX) blood concentration attained on any given day during exposure (1.09 mg/L; essentially a constant over 13 weeks as shown in Figure B2);

- the mid-point between the maximum (1.09 mg/L) and the minimum (0/mg/L) (MID) concentration on any given day during exposure (0.55 mg/L).

Using these values as potential dose surrogates in the human model, the model predicted air concentrations that would produce these steady-state concentrations in human blood with continuous exposure. As shown in Figures B3, B4, and B5, air concentrations predicted to attain these steady-state blood concentrations in humans with continuous exposure are 10.5 ppm (46 mg/m^3) for the TWA surrogate, 27.4 ppm (106 mg/m^3) for the MID, and 49.8 ppm (216 mg/m^3) for the MAX.

The rat model predicts that blood concentrations were essentially zero when the critical effects on rotarod performance were measured (24 hours after cessation of exposure). This supports the idea that the observed effects are not dependent on the concurrent presence of

xylenes in the blood and that they may be a persistent neurological effect. Brain concentrations would be a better dose surrogate to use in the model, but the model has not been developed to predict brain concentrations of xylenes.

The TWA dose surrogate is likely to provide a more accurate description of the exposure experienced by the rats in the study than do the MID or MAX dose surrogates, especially since the effects were measured after m-xylene had completely cleared from the blood. Thus, using the TWA as the dose surrogate in extrapolating from the rats in the Korsak et al. (1994) study to humans, the model predicts a HEC of 46.5 mg/m^3. This is very similar, although not identical, to the HEC (39 mg/m^3) predicted using the default $NOAEL_{[HEC]}$ dosimetry methodology, and it provides support for the RfC derived in Section 5.2.3.1.

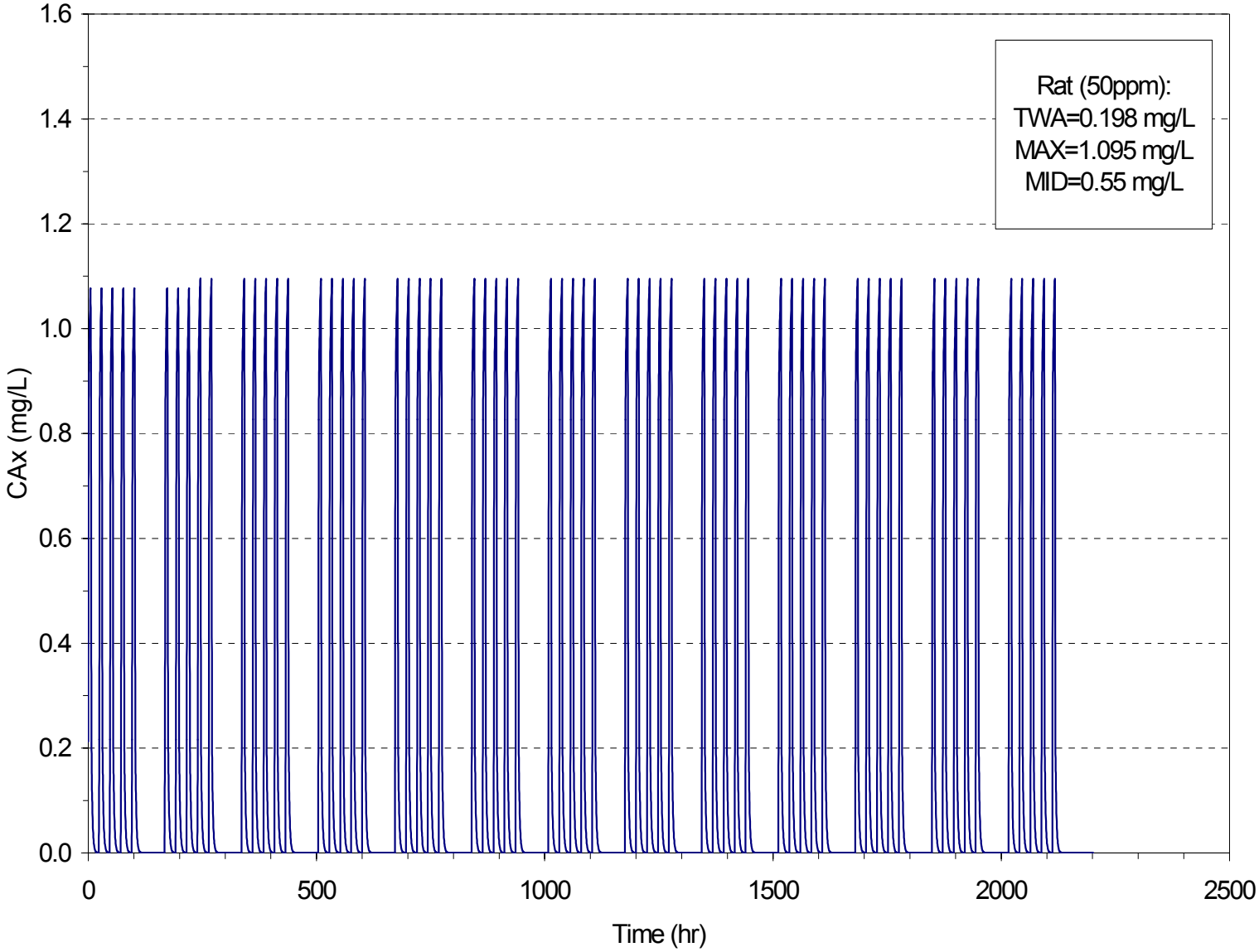

Rat (50ppm):
TWA=0.198 mg/L
MAX=1.095 mg/L
MID=0.55 mg/L
CAx (mg/L)
Time (hr)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
500
1000
1500
2000
2500

Figure B2.

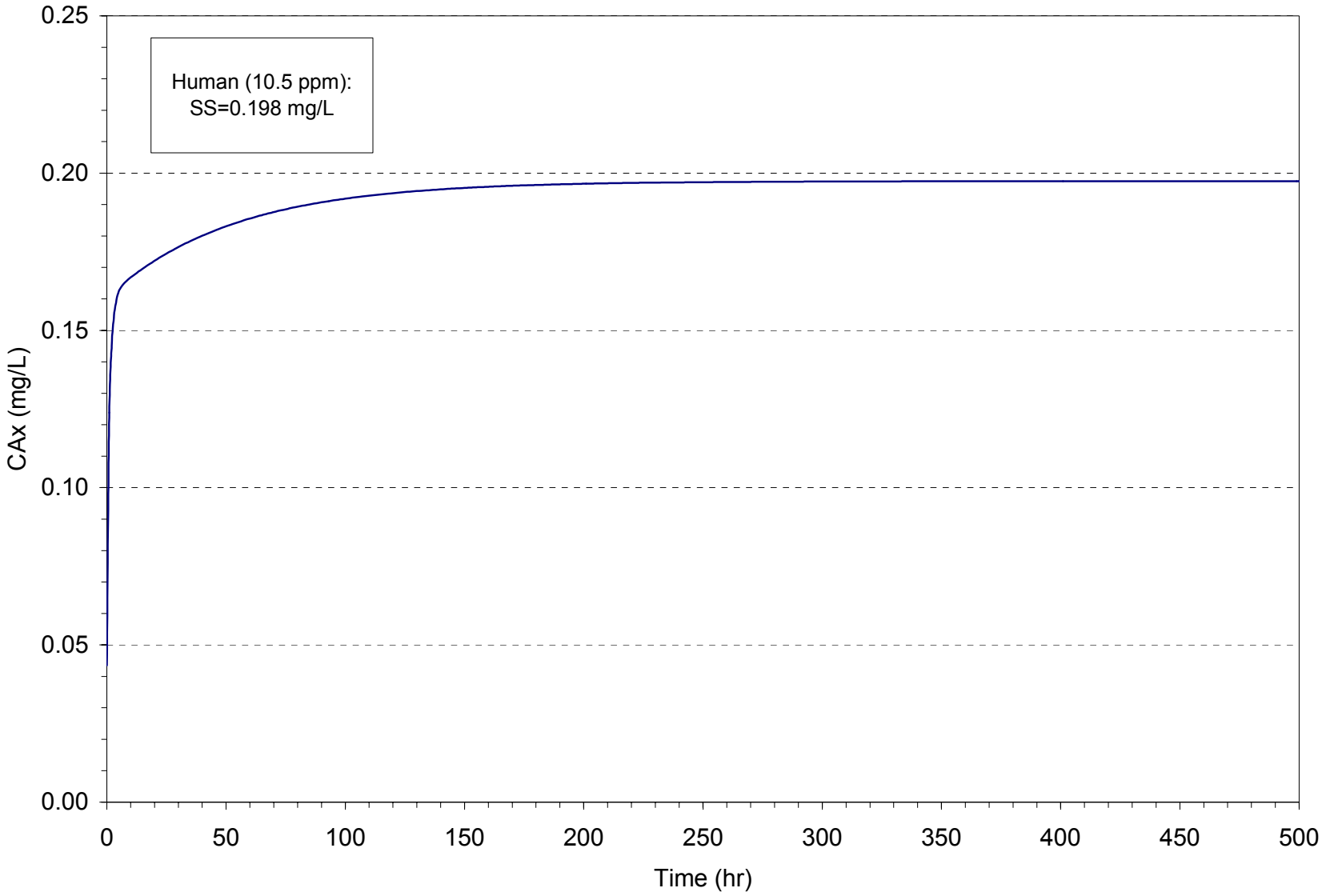

Human (10.5 ppm):
SS=0.198 mg/L
CAx (mg/L)
0.25
0.20
0.15
0.10
0.05
0.00
0
50
100
150
200
250
300
350
400
450
500
Time (hr)

Figure 11.

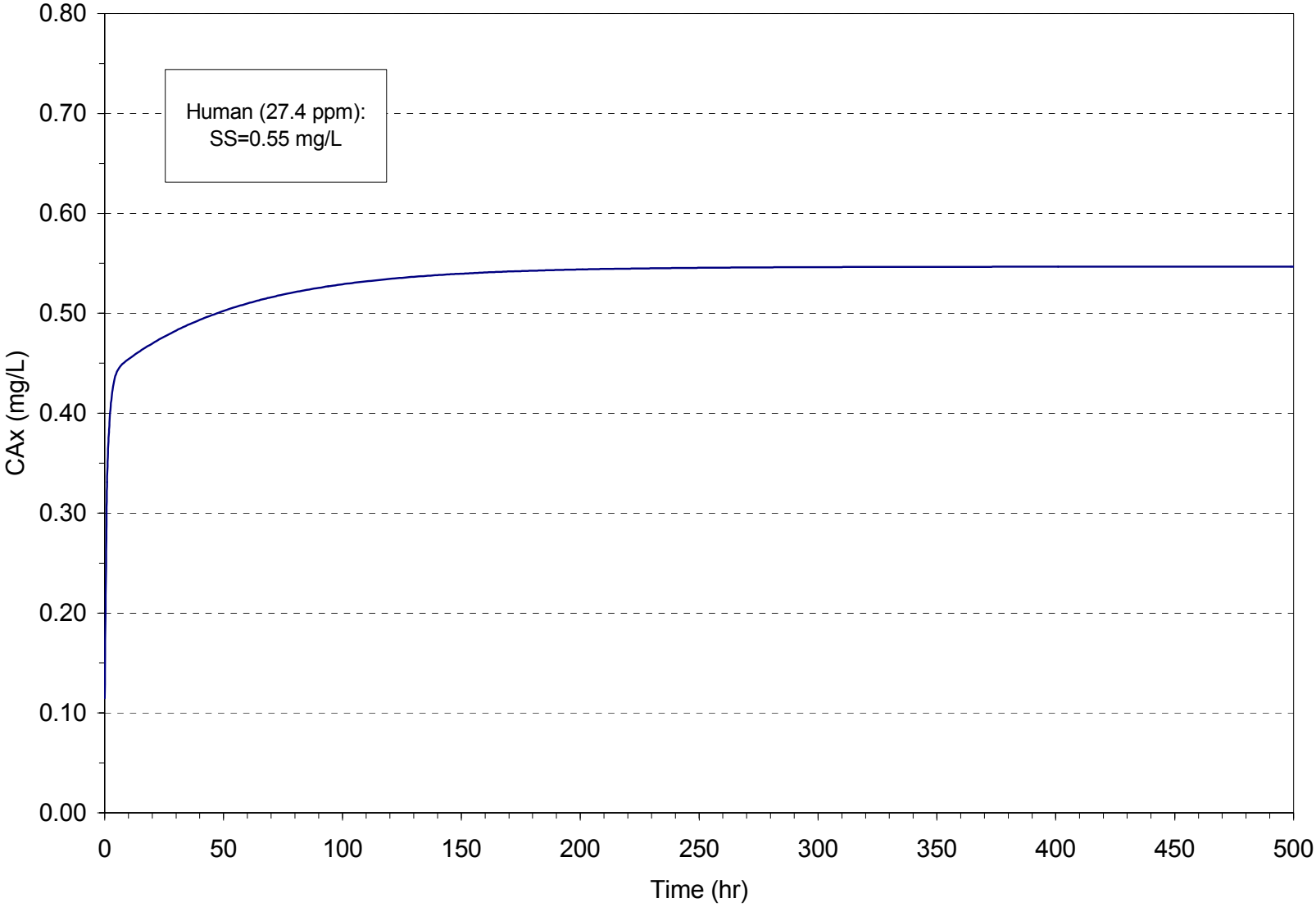

Human (27.4 ppm):
SS=0.55 mg/L
CAx (mg/L)
Time (hr)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0
50
100
150
200
250
300
350
400
450
500

Figure B4.

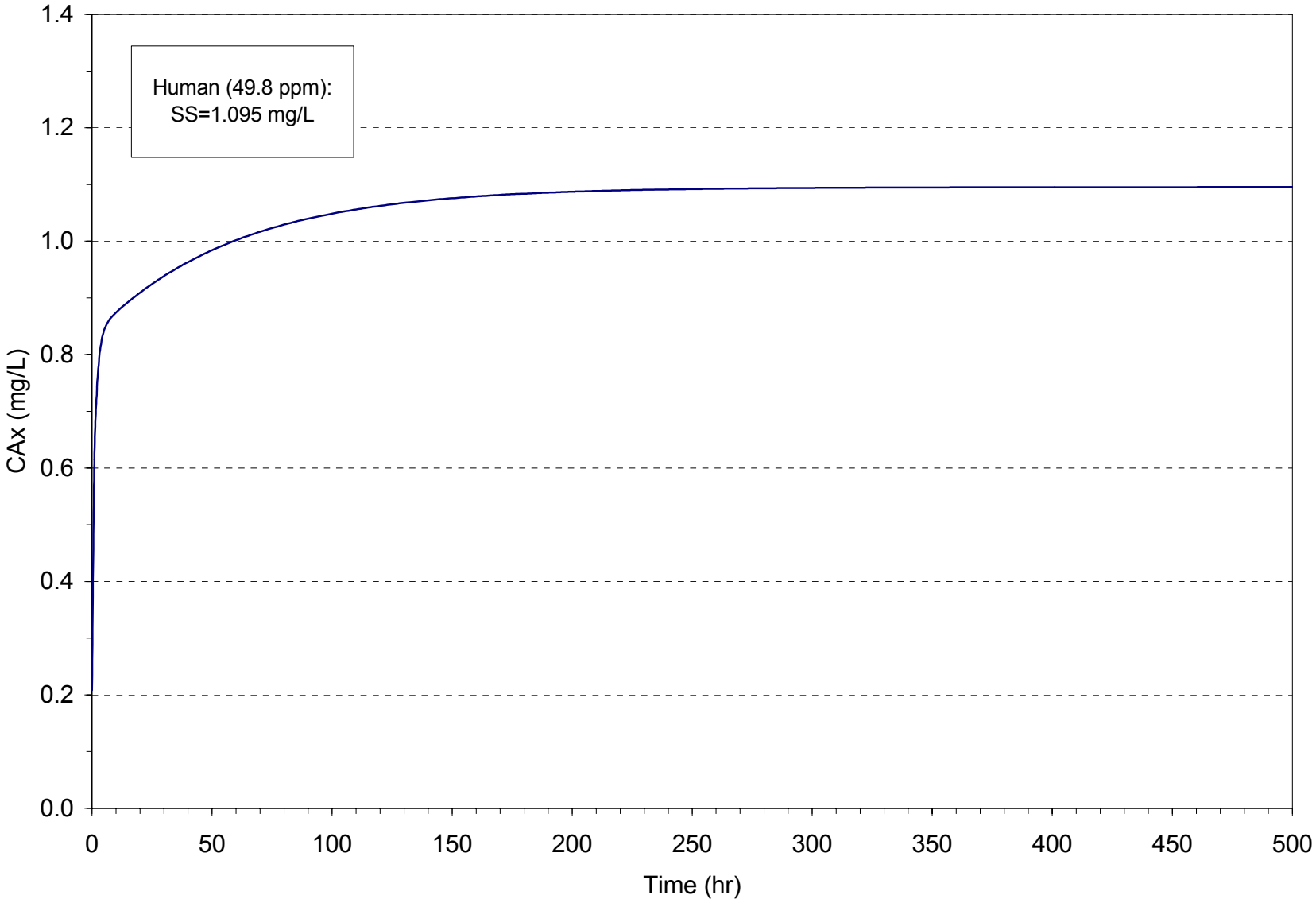
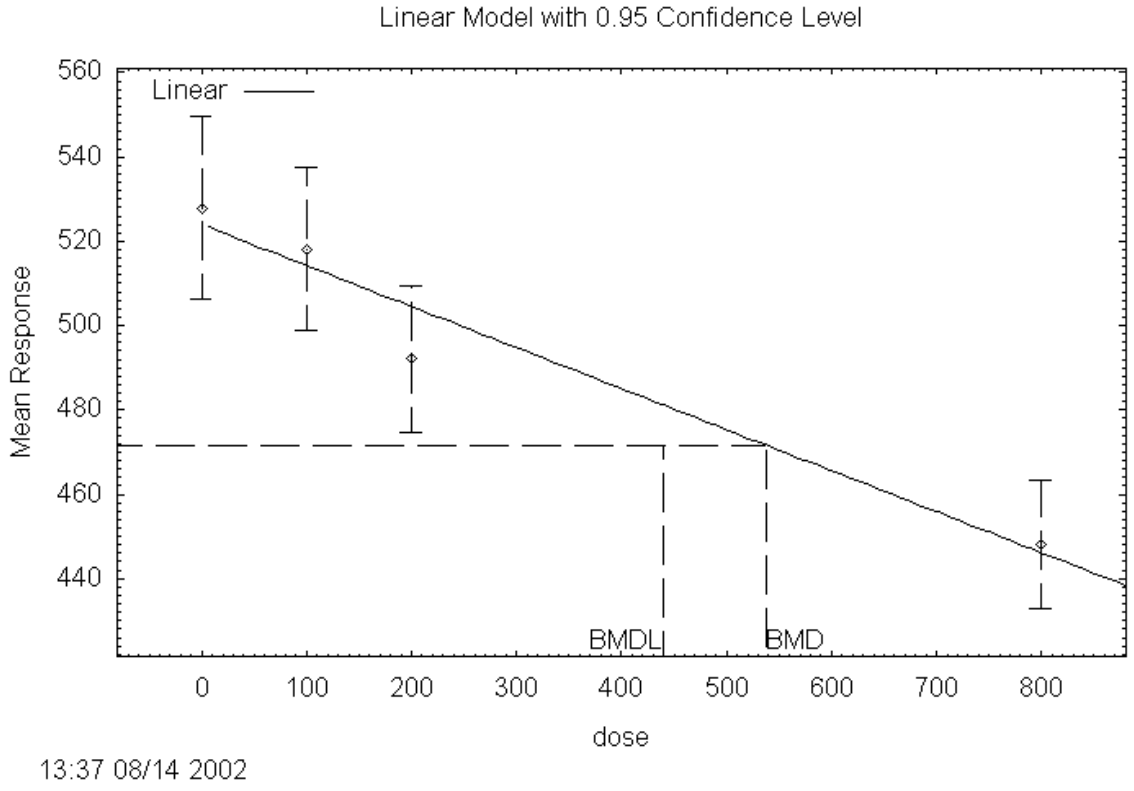

Human (49.8 ppm):
SS=1.095 mg/L
CAx (mg/L)
Time (hr)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
50
100
150
200
250
300
350
400
450
500

Figure B5.

APPENDIX C. BENCHMARK DOSE ANALYSIS OF WOLFE ET AL. (1988A)

The decrease in body weight in rats exposed orally to up to 800 mg/kg-day of m-xylene (Wolfe, 1988a) was analyzed using the models for continuous data in the EPA Benchmark Dose Software (version 1.3.1.). An appropriate fit was generated using a linear model; model outputs are included below. A 10% change in body weight was used as the benchmark response. The resulting BMDL value of 440 mg/kg-day is included for comparison purposes in the derivation of the RfD. Derivation of an RfD from this point of departure, using the same uncertainty factors (UFs) as noted in Section 5.1.3., but with an additional UF of 3 for extrapolation from subchronic to chronic duration (total UF of 3000), would result in a value of 0.1 mg/kg-day, which is similar to the RfD of 0.2 mg/kg-day in Section 5.1.3.



```
==========================================================================
        Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
        Input Data File: C:\EPA TOX REVIEWS\XYLENES\BMDS
STUFF\WOLFE\WOLFE_A_BODY_WEIGHTS.(d)
        Gnuplot Plotting File:  C:\EPA TOX REVIEWS\XYLENES\BMDS
STUFF\WOLFE\WOLFE_A_BODY_WEIGHTS.plt
                                                Wed Aug 14 13:37:50 2002
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


  Dependent variable = MEAN
  Independent variable = Dose
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008



            Default Initial Parameter Values
                  alpha =           1
                    rho =           0   Specified
                 beta_0 =     523.115
                 beta_1 =   -0.0966903
```

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	1364.87	230.706
beta_0	523.849	5.81841
beta_1	-0.0972985	0.013886

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	1.8e-006	-1.4e-006
beta_0	1.8e-006	1	-0.65
beta_1	-1.4e-006	-0.65	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	20	528	46.3	524	36.9	2.14
100	17	518	37.5	514	36.9	1.83
200	15	492	31.3	504	36.9	-4.99
800	18	448	30.4	446	36.9	1.02

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(I) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(I) + e(ij)$
$Var\{e(ij)\} = Sigma(I)^2$

Model R: $Yi = Mu + e(I)$
$Var\{e(I)\} = Sigma^2$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-286.570013	5	583.140025
A2	-284.347816	8	584.695632
fitted	-287.658524	2	579.317047
R	-306.762705	2	617.525410

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	44.8298	6	<.0001
Test 2	4.44439	3	0.2173
Test 3	2.17702	2	0.3367

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 538.393

BMDL = 440.224