TOXICOLOGICAL REVIEW

OF

XYLENES
(CAS No. 1330-20-7)

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

January 2002

Notice

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U.S. Environmental Protection Agency
Washington, D.C.

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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 of other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager/Author

Chemical Manager

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

Authors

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

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

Reviewers

This document and summary information on IRIS have received peer review both by EPA scientist 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 Manager 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, Planning, and Evaluation, 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

Summaries of the external peer reviewer's 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 non-cancer 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 (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *the Revised Draft Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999); *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996); *Recommendation 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 Concentration and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

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-present), and GENE-TOX], and RTECS in conjunction with a comprehensive DIALOG search.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

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) (Fishbein, 1988; ATSDR, 1995). The exact composition of the isomers is commonly dependent upon 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-, and p-xylene and ethylbenzene (Fishbein, 1988). As such, most of the environmental and occupational exposures, and toxicological studies are conducted on this mixture of xylenes containing ethylbenzene. Other minor contaminants of xylene include toluene and C_9 aromatic fractions. 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 production capacity of mixed xylenes has been estimated to be 13.1 billion pounds, with 1990 and 1991 production estimates of approximately 6 billion pounds (ATSDR, 1995).

TABLE 1. PHYSICOCHEMICAL DATA FOR XYLENES

Parameter	Value	Reference
Synonyms	dimethylbenzene (1,2-; 1,3-; or 1,4-); xylol, m-xylene (m-isomer); o-xylene (o-isomer); p-xylene (p-isomer); methyl toluene	Budavari et al., 1996; ACGIH, 1991
CAS registry no.	1330-20-7 108-38-3 (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	ATSDR, 1995
Density	0.864 g/cm^3	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	Budavari et al., 1996
Solubility in water	practically insoluble; 130 mg/L	ATSDR, 1995
Log K_{OW}	3.12-3.20	ATSDR, 1995
Conversion factors in air	1 ppm = 4.34 mg/m^3 1 mg/m^3 = 0.23 ppm	Emergency and Continuous Exposure Limits for Selected Airborne Contaminants (1984)
Odor threshold in air (absolute)	0.7- 40 ppm	ACGIH, 1991

3. TOXICOKINETICS/TOXICODYNAMICS RELEVANT TO ASSESSMENTS

3.1 ABSORPTION

Data supporting the rapid absorption of xylenes into the body are based largely on the physicochemical parameters of xylenes. 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 researchers 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 retained or absorbed into blood, and would be expected to move from the blood into tissues in which neutral lipids predominate, such as adipose tissue, and possibly, less so with material that have charged substituents. The low water solubility of xylenes suggests that xylene would move into portions of tissues with a high lipid fraction. Accordingly, it 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.

There has been a considerable amount of work conducted on the uptake of xylenes by inhalation. Ogata et al. (1970) exposed human volunteers to either m- or p- xylene or a mixture of xylene and toluene in an exposure chamber for either 3 hours or for 7 hours with a 1 h 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 and, if so, at what level). During the last 2 hours of exposure the researchers determined 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 employed by the researchers. The authors noted that the methods used for this study were not state-of-the-art, but were employed to maintain consistency with earlier studies.

Direct measurements of the level of absorption of xylenes was conducted by subjecting volunteers to 200 or 400 mg/m^3 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 was consistent over time for all three isomers and ranged from 62.4% to 64.2% of the inhaled volume for concentrations reflecting a high solubility of xylenes in blood. Although the authors expected to see a decrease in the retention of xylene in the lungs over time, they found that the amount of xylene absorbed was constant over time (Sedivec and Flek, 1976a).

Riihimaki and Savolainen (1980) conducted studies on human subjects both at rest and during exercise to measure the kinetics and acute effects resulting from exposure to mixed xylenes. Healthy male subjects were exposed to xylene for 5 days, 6 hours per day with a one hour break at midday followed by 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. Additionally, 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) the retention consistently remained around 60% (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 is limited by the aqueous barrier. Overall, the lowest uptake rate was noted with the 100 ppm during sedentary activity (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 control the total uptake of xylenes are the ambient concentration of xylene and ventilation rates of the subjects.

In a similar study, Astrand et al. (1978) subjected volunteers to vapors of mixed xylenes. Volunteers were exposed to xylenes vapor for four periods of 30 minutes. Volunteers in the first group were exposed to 870 mg/m^3 vapors for 30 minutes at rest and 90 minutes during light exercise requiring 30% of the subjects' maximal oxygen uptake while the second group was exposed to 435 mg/m^3 with no activity for 30 minutes followed by three successive 30-minute step intervals of increasingly demanding workload requiring 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. In comparison with increasing workload, the retention started at about 65%, but dropped to 50% at the higher workloads and the corresponding increase with ventilation rate. Over the two-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 constant absorption rate of 64-65% for the first group and the fact that 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 estimate 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 a total of 30 experiments on 10 healthy volunteers (5 men and 5 women) between 17-33 years old (consisting of 3 measurements on each volunteer). The individuals were exposed to m-xylene vapor in an inhalation chamber at three concentrations (100, 300, 600 mg/m^3) for 8 hours with 2½ hour breaks. The investigators monitored the concentration of the m-xylene in the vapor, the ventilation, xylene retention, and the levels of m-methylhippuric acid excreted in the urine. In this study, the investigators found that the retention of m-xylene in the lung varied with the concentration of m-xylene and the duration of exposure. At 300 mg/m^3 the 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 the 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 from the 87% at the start to 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 for women was 272.3, 724.3, and 1359.2 mg m-xylene; and for men 341.9, 909.2, and 1711.6 for 100, 300, and 600 mg/m^3 exposures, respectively. The disparity between this study and others may be due to the use of the same subjects

David et al. (1979) conducted comparative studies on the uptake and metabolism of m-xylene by inhalation in humans and mice. 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 research involved studies on both humans and mice. 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 (the study provides no description of the level of activity or ventilation rates of the subjects). 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 saturation of the blood is not reached at these exposure levels.

There are limited studies of the absorption of xylenes by oral administration although none were found that allow for a quantitative measure of uptake via the oral route. As noted above, the physicochemical properties indicate that xylenes would be rapidly absorbed through the gastrointestinal tract into the body. Indirectly, other studies demonstrate uptake via the oral route through the detection of metabolites in the urine, or demonstrate uptake through effects arising from exposure to the chemical(s). For example, a study conducted by Bray (1949) employed either intubation or incorporated into the diet as methods of administration of either o-, m-, or p-xylene and measured the corresponding metabolites in urine. Additionally, other studies report effects resulting from ingestion of xylenes. Studies conducted by Condie et al. (1988) reported dose-related changes in liver and spleen weight, and changes in enzyme activity following oral administration of xylene. NTP (1986) conducted assays on mice and rats that elicited changes in liver weight and morphological indicators and significant neurological effects.

3.2 DISTRIBUTION

The $pK_{o/w}$ of xylene indicates that xylene is expected to move rapidly from aqueous solution (such as blood) into tissues containing a higher proportion of neutral lipids including 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 authors employed head space gas chromatography methodology to measure the relative affinity of the respective tissue relative to that of blood. The authors killed the rats and removed the organs of interest. The tissues were trimmed to remove extraneous tissue and to achieve uniform size after which the tissues 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 ethyl benzene and individual isomer of xylene in the head space was determined by gas chromatography. The xylene K_d for brain, muscle, and kidney are comparable and range overall from 1.5 to 3.7. The range of K_d include ranges in the administered 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 of all tissues tested with values ranging from 37 to 67. There was one value of 26 that appears to be an outlier. Overall, there were no differences across administered doses or between isomers.

In their study on the uptake and distribution of ethylbenzene and xylenes, Riihimaki 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 compared with venous blood following the last day of exposure (5 days exposure + weekend without exposure + 1 day of exposure).

Arstrand et al. (1978), in their study discussed above, found that following rapid uptake of xylenes vapors the concentrations increased in the arterial and venous blood. This was followed by an initially rapid but overtime, persistent loss of xylenes from the blood following cessation of exposure. Despite the rapid absorption of xylenes, the amount of xylenes found in the blood generally constituted 2% to 3% of the total xylenes absorbed. The authors postulate that these factors reflect the high lipid solubility of xylenes resulting in the distribution to 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 xylenes. As with the Astrand et al. (1978) study, these researchers subjected volunteers to either 870 mg/m^3 of xylenes vapors during a 30-minute resting period followed by light exercise, or 435 mg/m^3 of xylenes 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 authors found that 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. The data may also reflect a redistribution from tissues

with a higher water content or with ionically-charged lipids to tissues with a higher proportion of neutral fat.

Additional information on the distribution of xylenes in the body are 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. At the end of the exposure, the rats were removed from the inhalation chamber, treated with anaesthesia, and returned to the chamber to avoid loss of xylene. After the anesthesia had taken affect, a blood sample was taken from the aorta, the animals were dissected, and the kidney, liver, brain, and fat were harvested. The tissues were subjected to head space-gas chromatography analysis. Head-space samples were taken and analyzed 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 with fat containing 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 its peak concentration following the 8-hour exposure (270 ± 7 nmol/g tissue). For the remaining tissues, the relative tissue m-xylene concentrations were ischiadic nerve> blood=liver≥ lungs> cerebrum= cerebellum= muscles= spleen. Concentrations of xylene-equivalents in the cerebellum, cerebrum, muscles, spleen, and lunges paralleled the concentrations of xylenes in the arterial blood throughout the entire exposure period. The distribution of xylenes in tissues parallels that seen in the study by 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, while 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, the liver up to 2 hours, in the bile from 2 to 8 hours, in the nasal mucosa and bronchi from 2 - 24 hours, and in the adrenal medulla immediately after (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

Ogata et al. (1970), in the study previously described, exposed human volunteers to differing concentrations of either m- or p-xylene for varying periods of time. The data demonstrated a linear relationship between the exposure to xylenes either in concentration or

over a period of time, and the amount of methylhippuric acid excreted in the urine as measured over an 18-hour period. These data also demonstrated that the rate 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.

Riihimaki and Savolainen (1980), in their study of inhalation exposure of xylene to human subjects under sedentary and physically active conditions found that 95% of the absorbed xylene was converted to methylhippuric acid with the remainder lost as nonmetabolized xylene in expelled air.

Riihimaki (1979) conducted a study to evaluate the metabolism and excretion of xylene and toluene derivatives by a human volunteer. The researcher administered doses to a single individual of acid derivatives of xylene and toluene (methylbenzoic acid and benzoic acid, respectively) or the conjugates (methylhippuric acid or hippuric acid, respectively) and measured the excretion of the metabolites in the urine. The subject was administered a single dose of 7.4 mmole m-methylbenzoic acid, 41 mmole benzoic acid, 7.8 mmole m-methylhippuric acid or 33.5 mmole hippuric acid. In a combined dose study, the same individual was administered 7.4 methylbenzoic acid followed by 41 mmole benzoic acid, and 7.8 mmole methylhippuric acid followed by 33.5 mmole hippuric acid. The urine was analyzed for 30 hours following administration for the presence of metabolites. All the administered toluene or xylene derivatives appeared in the urine as hippuric acid or methylhippuric acid, respectively, indicating that, under the conditions of this study, once the xylene has been oxidized to methylbenzoic acid, the only route of metabolism, for the doses administered, was as the glycine conjugate. The administration of both the benzoic and methylbenzoic acid demonstrates that the two chemicals compete for the available stores of glycine. Consideration of the relevance of metabolism to the rate of excretion is discussed below.

Some of the earlier studies on the metabolism of xylenes were conducted by Bray et al. (1949) in which rabbits were intubated and fed either o-, m-, or p-xylene or the corresponding toluamide or toluic acid, and analyzed for metabolites in the urine. The authors relied on rudimentary colorimetric methods. The investigators found higher levels of the glucuronide metabolite in urine of rabbits that were force fed the toluic acid metabolite and relatively small amounts of the glucuronide with the xylene. The authors concluded that xylenes are metabolized to the toluic acid which is subsequently conjugated with glycine of which the rate limiting step is the enzymatic conversion of xylene to the acid. In the absence of sufficient amounts of glycine, as with the bolus administration of toluic acid, the acid reacts with other possible reactants. The authors note that there is 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, the authors found that with bolus administration of the xylenes, most of the chemical is excreted as either the acid or glycine conjugate (i.e., methylhippuric acid).

In comparison with the findings of Bray et al. (1949), Sedivec and Fleck (1976a) in a study mentioned above, measured the production of conjugates of toluic acids in the urine of subjects exposed to 200 and 400 mg/m^3 of individual isomers of xylene vapor. The authors

found that all of the toluic acid derivatives were in the form of glycine conjugates (hippuric acid and methylhippuric acid), but found no evidence of glucuronic conjugates. They attributed this finding to the method of dosing in which a bolus application of 0.6 g/Kg in the Bray et al. (1949) study compared with their estimated administration by the inhalation route of 0.019 g/Kg. While they acknowledge that the effect may be species specific (rabbits and humans), they felt that the dosing was the decisive factor. They also determined that in addition to the methylhippuric acid, xylenols were formed at considerably lower concentrations than the methylhippuric acids.

This effect was also seen in studies conducted by Ogata et al. (1980). The researchers conducted studies under three different scenarios: IP injection of xylenes into rats (11.3 mmol/kg); oral administration in human volunteers (0.368 and 0.736 mmol/kg); inhalation exposure to human volunteers (138 ppm for 3 h) followed by analysis of urine over time. The results demonstrate that at the highest doses (e.g. rats at 11.3 mmol/kg ip xylene) the relative amount of toluic 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 toluic glucuronide. One explanation may be differences in the toxicokinetics between species. On the other hand, it could reflect differences arising from routes of administration as the rats received the IP administration which led to the saturation of enzyme systems in the rat or depletion of glycine stores. The lowest dose was through inhalation which occurred over 3 hours as opposed to bolus administration by IP or oral ingestion. The administration by IP injection of o-xylene into rats reflected the highest amount and proportion of nonmetabolized xylene in the urine of the three scenarios. While 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), 5 male and 5 female volunteers were exposed to 3 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. As such, there was no measurement 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 in these studies was converted to methylhippuric acid.

Astrand et al. (1978) subjected volunteers to two concentrations of mixed xylene vapor and measured the persistence of xylene in the body. The two procedures involved the absorption of xylenes at 870 mg/m^3 during resting for 30 minutes followed by 90 minutes of light exercise, and 435 mg/m^3 during 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 of xylenes expelled through the lungs. They found that following cessation of exposure the amount of xylenes in the arterial and venous blood decreased rapidly. Five per cent and four per cent of the xylenes absorbed during exposure for the resting followed by light exercise and resting with variable exercise exposures, respectively, of the absorbed xylenes were lost through the lungs as nonmetabolized xylenes. The remainder was presumed to be excreted in the urine. The authors attribute the rapid loss to metabolism of the xylenes (although they did not measure the

production of methylhippuric acid or the amount of xylene in the urine). Despite the rapid initial loss 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 the high solubility of xylenes for the lipid component in tissues. As the xylenes in the blood decreases 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 his study mentioned above, Carlsson (1981) measured the distribution of xylene as compared with its metabolites. During the exposure, the highest percentage of metabolized xylene (mostly methylhippuric acid) was found in liver and blood that contained 60%-70% of the xylene equivalents as metabolites while subcutaneous fat had the lowest percentage (20%). Three hours following cessation of exposure, the concentration of xylene equivalent in the metabolite form increased from 50 to 67%, to about 95% in the liver. However, in the muscle the percentage of xylene equivalents in the metabolite form ranged from 61 to 71% during exposure which subsequently decreased to 40% of the xylenes in the metabolite form at three hours following exposure. In subcutaneous fat the relative concentration of xylene equivalent in the metabolite form remained constant at about 20%.

3.4 EXCRETION

Most of the xylene taken up by individuals is lost in the urine in the form of glycine conjugates of the benzoic acid as noted above.

In the study by Riihimaki (1979) (described above) a single volunteer was administered individually benzoic acid derivatives of toluene and xylene followed by a measurement of the rate of loss of the chemicals and the form in which it was taken. As noted above, all of the administered acid was excreted in the form of glycine derivatives (i.e., hippuric acid and methylhippuric acid for benzoic acid and methylbenzoic acid, respectively). However, the rate of loss was greater for the methylhippuric acid metabolite treatment compared with the methylbenzoic acid treatment. The data indicate that all of the administered metabolite was excreted in the urine, but in comparison with the administered glycine conjugates the acid was excreted at a slower rate. These data indicate that the rate-limiting step for the excretion of xylene is the conjugation of methylbenzoic acid with glycine. This study is limited by the fact that it was conducted on a single individual. The determination that the loss of xylene is limited by the availability of glycine suggests that the rate of loss of glycine may vary with such factors as age and nutritional status of the individual.

Riihimaki and Savolainen (1980) exposed volunteers to either constant levels of 100 ppm or 200 ppm, or fluctuating concentrations with peak concentrations of 200 ppm or 400 ppm xylene, either in sedentary state or with intermittent periods of exercise on an ergonomic bicycle. The authors report that 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. Xylene in adipose tissue can be expected to take longer to leave owing to the greater solubility of xylene in fat than in blood which accounts for the presence of low levels of xylene in the blood for several days following cessation of exposure. The authors propose that the two phases representing the rapid loss of xylene from the blood, mostly through conversion to methylhippuric acid followed by excretion, indicates that well-perfused organs reach equilibrium within minutes, muscles reach equilibrium within a few hours, while adipose tissues 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 for loss of xylenes in the expired air and metabolites in urine for periods initially of 10-minute intervals, subsequently 30-minute intervals, and, on the second day, 4- and 8-hour intervals for 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 h following termination of exposure. Even on the second day there were detectable amounts of nonmetabolized xylene in the expired air. 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 with the p- isomer relative to the other two. When administered as a mixture of isomers, 4.8% of the absorbed p-xylene was lost through the

lungs. The loss of nonmetabolized xylene in the urine appeared 2 h following the beginning of exposure, but remained low throughout the study.

The authors measured the loss of xylenes in urine as nonmetabolized xylene, toluic acid, and toluric acid (methylhippuric acid). Analysis of fresh urine did not detect any toluic acid. However, following storage at ambient temperatures for "several days" toluic acid appeared in urine samples from the same sampling. The authors attribute this to the enzymatic hydrolysis of methylhippuric acid to the acid and the amine, mediated by microbial contamination. In other studies glucuronic acid has been detected. The authors state that with their analytical capabilities they were unable to directly measure the amount of glucuronic acid in the urine but noted that since the amount of toluic acid in the aged sample was the same as the methylhippuric acid in the fresh urine they presumed that no glucuronic acid or other conjugates were produced. While differences between this study and others demonstrating the presence of glucuronic acid conjugates in rabbits following xylene exposure may be due to differences in metabolism of species, the authors feel that dosing regimen is the decisive factor. In the Bray et al. (1949) study, the researchers administered 0.6 g/kg compared with the Sedivec and Flek (1976a) study in which the animals adsorbed 0.019 g/Kg xylene or 30x lower. Overall, loss of nonmetabolized xylene in the urine accounted for less than 0.005% of the amount of xylene retained in the system.

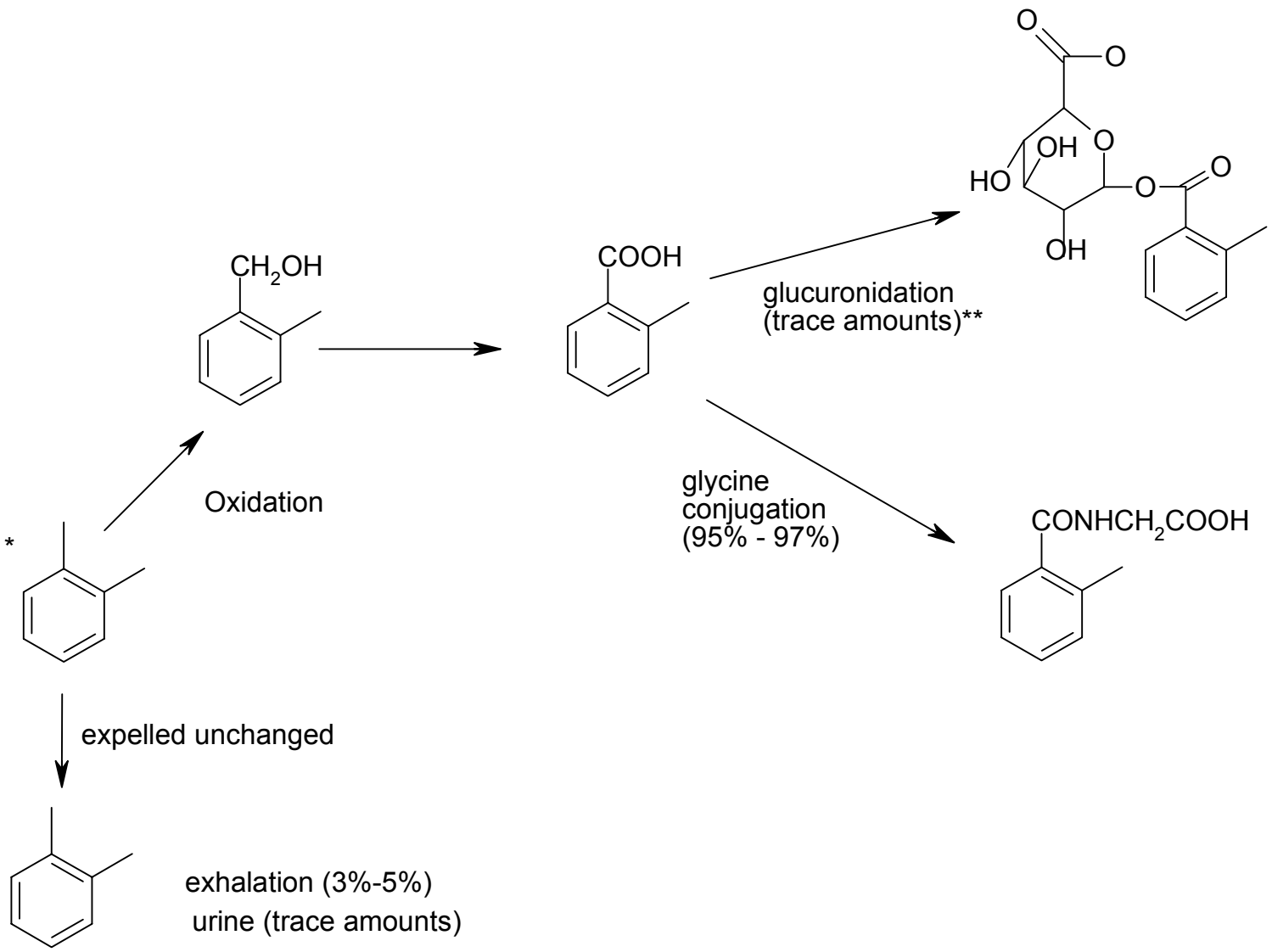
In addition to monitoring the loss of nonmetabolized xylene in expired air and urine, and the corresponding acid or glucuronic acid, Sedivec and Flek (1976a) tracked the course of excretion of the methylhippuric acid following inhalation exposure. They detected methylhippuric acid in the urine following 2 hours of exposure which increased during exposure and reached the highest concentration 2 hours following termination of exposure. Following the 2-hour post exposure sampling, the amount of methylhippuric acid in the urine decreased rapidly, but was still detectable in the urine 4-5 days following the exposure. There were no significant differences in the amount of methylhippuric acid excretion between the three isomers when exposed individually; however, when exposed in a 1:1:1 ratio, the authors found that p-toluic acid derivatives constituted a larger percentage (41.5%) compared with m-toluic acid (34.7%) and 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, the methylhippuric acids accounted for 97.1%, 99.2%, and 95.1% of the absorbed o-, m-, and p- isomers, respectively, and for xylenols 0.86%, 1.98%, and 0.05%, respectively.

For the most part, recent studies have used the level of methylhippuric acids in the urine as a quantitative indicator of respective xylene. Sedivec and Flek (1976b) measured the amount of "toluic acids" (methylhippuric acids) in urine as an indicator of exposure. The authors exposed human volunteers to 200 or 400 mg/m^3 for 4h or 8h with a 2 h break. The authors found that 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 to 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.

In the aforementioned study by Carlsson (1981), the amount of xylene equivalents (xylene and its metabolites) in male rats exposed to 208 mg/m^3 ^{14}C- p-xylene for 1-, 2-, 4- and 8-hour exposure. The authors found that following rapid absorption of the xylene and its broad distribution throughout the body there was a rapid loss from the various tissues. However, 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 one 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 the high lipophilicity and hence its high $K_{o/w}$.

In their study, David et al. (1979) tracked the loss of m-xylene through the urine in the form of methylhippuric acid in humans exposed to 400 mg/m^3 m-xylene. The methylhippuric acid metabolite first appears in the urine within two hours of the start of the exposure and reaches 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 still detectable but low 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 three days prior to the start of the study. The rats were exposed to m-xylene concentrations ranging from 400 to 2,000 mg/m^3. The researchers found that at exposure concentrations above 800 mg/m^3, prior treatment of the rats pretreated with phenobarbital demonstrated an increased ability to metabolize xylene. These levels exceed long term exposure limits and therefore humans are not expected to be exposed to this concentration.



* 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
based on 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; Senczuk and Orlowski, 1978; David et al., 1979

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS — EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1 Cancer Studies

Arp et al. (1983) and Wilcosky et al. (1984) both reported the results of a reanalysis of a cohort of rubber industry workers. The exposures of the workers were reconstructed using company records. The objective of the reanalysis by Arp et al. (1983) was to study the relationship between workers who were occupationally exposed to benzene and other solvents, and lymphocytic leukemia. The cohort from which the cases and controls were identified was defined as all active or retired hourly workers, age 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 the associated activities which were used to estimate the potential for exposures. The authors provide no indication of the exposure levels. The authors employed titles and longevity in the position to estimate periods of exposure to specific solvents. Solvent composition was inferred from records of formulations of raw materials. The time periods for which the cohorts were exposed are important because of changes in the production of solvents from coal-based to petroleum based sources 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 considers exposure to benzene and to secondary solvents that includes xylenes.

The odds ratios for lymphocytic leukemia from exposure to solvents other than benzene, that includes xylenes, was 4.50 ($p = 0.08$) (Arp et al., 1983). However, when these values were broken out into coal-based xylenes the odds ratios are 5.50 ($p = 0.02$, 6 cases) compared with all petroleum-based solvents (principally xylenes) (OD = 1.50, $p = 0.41$, 11 cases). The authors do not provide data for 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) compared with exposure to petroleum-based solvents (OD = 1.50, $p = 0.41$, 11 cases). The authors note that the source of commercial aromatic solvents changed during the 1940s from coal-based solvents to petroleum-based solvents. The source of the solvents indicates the composition of contaminants in the mixture. Petroleum-based solvents typically have straight-chain aliphatic alkanes while 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. The authors note the following shortcomings of the study: an inability to accurately measure the exposures to workers; the composition of the solvents; the small number of cases employed to derive the odds ratio; and that there may not have been a sufficient latent period for the development of tumors arising from the cohort exposed to petroleum-based solvents.

Wilcosky et al. (1984) conducted a reanalysis employing the methods described by Arp et al. (1983) and using the same cohort for this study as the Arp et al. (1983) study. This study also employed workers of the same age 40 to 79 for the same ten year period. As with the Arp et al. (1983), study exposures were estimated based on occupational titles. A cohort was considered exposed when holding a title that involved direct contact with a chemical for 12 months. In the Wilcosky et al. (1984) study, exposures are broken down into individual chemicals. The authors found a statistically significant increased odds ratios of 3.7 ($p < 0.05$, 4 cases) for lymphosarcoma and a nonstatistical increase (OD = 3.3, 4 cases) for lymphatic leukemia resulting from exposure to xylenes. Interestingly, despite the findings in the previously described study (Arp et al., 1983) the authors of this study did not break out those exposed to coal-based solvents and petroleum-based solvents. The authors identify issues with this study such as the findings of a negative correlation between solvent and lung cancer and the limited number of cases for each, and the authors admonish readers of the need for "guarded conclusions" based this study.

In brief, while the entire cohort was comprised of 6678 workers, the number that was 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 odds ratio for lymphosarcomas (3.7; $p<0.05$) and a nonstatistical increase for lymphatic leukemia (3.3) in workers exposed to xylenes. Only 4 cases were found for each.

Spirtas et al. (1991) conducted a retrospective cohort mortality study of 14,457 workers who worked for at least one 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 conducted 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 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 person-years of the workers exposed to xylene was small (1837 for males and 444 for females), the composition of the 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.

Gerin 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 individuals in hospitals suffering from incidence of cancer. The questions involved information on the lifestyles and work habits of the patients. This information was used to identify potential exposure to benzene, toluene, xylene, and styrene which were semiquantitatively categorized into low, medium, or high exposure. The researchers employed randomly selected individuals to serve as controls. Although an increased odds ratio was reported for exposure to "high" concentrations of xylene and cancer of the colon (5.8; 8 /

429 in the case compared with 3 / 955 for the controls) and rectum (SMR = 2.7; 5 / 213 in case compared with 8 / 937 in 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 since the authors note that statistically-significant associations were found between each of the four compounds and rectal cancer. The authors found a high correlation in the exposure pattern and a likely high correlation in errors of exposure and therefore concluded that the results should be interpreted cautiously.

4.1.2 Noncancer Studies

4.1.2.1 Cohort Studies

Surveys conducted in factories in China identified workers exposed to solvents in the production of rubber boots or plastic coated wires, or in printing work (Uchida et al., 1993). The survey identified 994 solvent-exposed workers. To identify and quantify solvent exposures, the workers were equipped with a diffusive sampler for an entire 8-hour working shift. A total of 175 xylene-exposed workers (107 men, 68 women) were selected for the study "for whom the sum of the three xylene isomers was such that the sum of the three isomers accounted for 70% or more of the total solvent exposure (on a ppm basis)." The next day, these workers underwent a medical examination that included subjective symptoms, clinical signs, 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 in their working life, were of similar ages, and had comparable drinking rates and smoking habits. Quantitative health measurements consisted of hematology, serum biochemistry, and urinalysis. Subjective symptoms were evaluated by means of a survey inquiring about symptoms experienced during the work shift, and another survey recording 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, while benzene was never detected. There was little difference in the amount of solvent exposure between men and women.

The prevalence of subjective symptoms during the work shift and in the previous three month period was significantly higher ($p<0.01$) in exposed workers compared to 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 in both sexes, while 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, while poor appetite was the only symptom reported for the previous 3 months that was dependent upon concentration. No significant differences in measured hematology, clinical biochemistry, or urinalysis parameters were noted in exposed workers compared with controls. Therefore, no effect level was identified.

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. The first patient 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. The second patient 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 three 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. Patient 3 recovered consciousness following admission, and 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 a suicide committed by a 27-year-old man from the ingestion of 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, accidental ingestion of xylene resulted in a deep coma lasting over 26 hours, hepatic impairment, hematemesis, acute pulmonary edema, and other pulmonary complications (Recchia et al., 1985). Another individual attempting to commit suicide by the intravenous injection of 8 ml of xylene developed acute pulmonary failure within 10 minutes of administration (Sevcki 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 reported in the literature. Goldie (1960) reported a case where 8 painters were exposed to paint containing 80% xylene and 20% methylglycolacetate. The workers complained of headache, vertigo, gastric discomfort, dryness of the throat, and slight drunkenness after 30 minutes of exposure. After two months of exposure, an 18-year-old worker exhibited behavior indicative of a convulsive seizure. Signs included weakness, dizziness, inability to speak, unconsciousness, eyes and head rotated to one side, chewing but no foaming, and exhibited kicking motions. The subject recovered consciousness 20 minutes later. In another case, Arthur and Curnock (1982) reported 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 provide an exposure concentration, and exposures were not limited to xylene alone.

Klaucke et al. (1982) reported that fifteen employees who had been exposed to xylenes were admitted to a small community hospital each complaining of at least two of the following symptoms: headache, nausea, vomiting, and dizziness or vertigo, eye irritation, or nose or throat irritation. The frequency of the symptoms were as follows: 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 workers were exposed to levels as high as 700 ppm.

Five women occupationally exposed to xylene from 1.5 to 18 years experienced symptoms including chronic headache, chest pain, ECG 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. Oral studies

Subchronic oral studies in rats do not consistently implicate a single organ system. Effects, where noted, include nephrotoxicity, increased mortality possibly related to compound inspiration following gavaging, decreased body weight gain, increased salivation, and hepatic changes evident only by electron microscopic evaluation. These studies are described below. Neurotoxicity was seen in studies in mice and rats: these studies are discussed in Section 4.4.1.2.

Groups of 10 male and 10 female Fischer 344 rats were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 17.0% ethylbenzene, and 9.1% o-xylene) in corn oil by gavage at doses of 0, 62.5, 125, 250, 500, or 1000 mg/kg/day for 5 days/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 than controls, respectively, after 13 weeks of exposure, with final body weights being 89% and 97% of controls, respectively (statistical significance not reported). No signs of toxicity or treatment-related gross or microscopic changes were observed. The LOAEL was 1000 mg/kg/day based on decreased body weights in males, and the NOAEL was 500 mg/kg/day.

In the same study (NTP, 1986) male and female $B6C3F_1$ mice were treated with mixed xylenes. Groups of 10 mice of each sex were administered 0, 125, 250, 500, 1,000, and 2,000 mg/kg/day in corn oil by gavage, 5 days/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 2,000 mg/kg/day, starting 5-10 minutes after dosing and lasting for 15-60 minutes, the animals exhibited lethargy, short and shallow breathing, unsteadiness, tremors, and paresis. Mean body weight of the mice in the high dose group was 7% lower than the vehicle control for males and 17% lower for females. While not stated explicitly, the text implies that this was a common finding among the animals dosed at this level. There was no treatment related gross or microscopic pathologic lesions seen in this study. The NOAEL is 1,000 mg/kg/day and the LOAEL is 2,000 mg/kg/day for neurotoxic effects.

In a study by Condie et al. (1988) groups of 10 male and 10 female Sprague-Dawley rats were administered mixed xylenes (o-xylene: 17.6%; m - and p-xylene (which coeluted); 62.3%; ethyl benzene: 20%) by gavage in corn oil for 90 consecutive days at dosages of 0, 150, 750, or 1500 mg/kg/day. Effects of exposure included decreased body weights in high-dose males (86% of controls); dose-related increased liver weights and liver-to-body weight ratios in mid- and high-dose males (17 and 37% of controls) and females (14 and 30% of controls); and increased kidney weights and kidney-to-body weight ratios in mid- and high-dose males (14 and 27% of controls) and high-dose females (18 and 16% of controls, respectively). The authors postulate that the modest increases in aspartate aminotransferase in high-dose females and alanine aminotransferase in high-dose males and mid-and high-dose 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). The LOAEL is 750 mg/kg/day based on increased kidney weights and early appearance of nephropathy in females, and the NOAEL is 150 mg/kg/day.

Of the studies in which mixed xylenes were administered by the oral route, Condie et al. (1988) is the only study which identified detectable kidney effects other than changes in weight. Wolff (1988a, b), NTP (1986), and Borriston Laboratories Inc. (1983) conducted studies on xylenes exposure via the oral route in rodents without detecting chemical related nephrotoxic effects relative to the controls. Condie et al. (1988) characterized the kidney effects as "minimal."

In a study by Bowers et al. (1982), aging (12-19 months) Long-Evans hooded, male rats were fed methylated benzenes, including o- xylene, incorporated into the feed at a concentration of 200 mg/kg feed (10 mg/kg/day) for 1, 2, 3, or 6 months for assessment of ultrastructural changes in the liver. No other endpoints were evaluated. Although the liver was grossly normal, electron microscopic evaluation of the liver revealed two types of membrane-bound vacuoles in hepatocytes from rats fed xylene appearing 1 month after 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 rates for the 0, 100, 200, or 800 mg/kg/day groups were 20/20, 17/20, 15/20, and 18/20 for the males, respectively, and 20/20, 20/20, 16/20, and 16/20 for females, respectively. Mortality in the mid-dose males and mid- and high-dose females attained statistical significance ($p \leq 0.05$), 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 was 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 ($p \leq 0.05$) in mid- and high-dose males (89% and 75% of controls, respectively) and high-dose females (85% of controls). Food consumption was likewise decreased ($p \leq 0.05$) in high-dose males during weeks 1-5 (90%) and in mid- and high-dose males during weeks 6-9 (92% for both). Other effects noted were not definitively related to treatment and/or were not biologically significant. The authors identified the NOAEL and LOAEL at 200 mg/kg/day and 400 mg/kg/day, respectively, based on decreased body weight. There were no abnormal histopathology findings including kidney effects.

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 rates for the 0, 100, 200, or 800 mg/kg/day groups were 20/20, 19/20, 17/20, and 16/20 for the males, respectively, and 20/20, 18/20, 18/20, and 17/20 for females, respectively. Mortality in high-dose males attained statistical significance, and a statistically significant trend for mortality 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 one-hour post dosing in both high-dose males and females. Body weight gains at 13 weeks were slightly reduced (89%, not significant) in high-dose males and females, while 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 in organ weights. Histopathology revealed no abnormal findings in any tissue or organ. Authors of the study identify a NOAEL of 200 mg/kg/day and a LOAEL of 800 mg/kg/day based on reduced body weight and mortality.

The two studies by Wolfe (1988a; b) identify NOAELs of 200 mg/kg/day; however, they reveal no overt toxicity findings for the parameters measured given the conditions of the study. The author believes that the cause of the mortality was related to aspiration; however, the cause of the aspiration is unclear.

Groups of 10 male Fisher 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 in a nephrotoxicity screening study (Borriston Laboratories, Inc., 1983). No nephrotoxic effects were observed in the rats dosed with m-xylene as compared with the controls.

4.2.1.2. Inhalation studies

Of the available inhalation studies that examine systemic effects no consistent endpoints of toxicity were identified. Effects, when noted, primarily include mild hepatic changes, changes in the vascular tone of microvessels, and respiratory paralysis.

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; 19.3% ethyl benzene; 7.8 % p-xylene; 7.6% o-xylene) for 6 hours/day, 5 days/week, for 65 or 66 days for the two species, respectively. Endpoints assessed in the rats and dogs included changes in body weights, hematology, clinical chemistry, urinalysis, organ weights, and histologic examination. Three rats from each dose level were also killed after 15 and 35 days of exposure for histologic evaluation. Additional measurements in dogs included food consumption, and initial and terminal electrocardiograms. No treatment-related effects in any of these measured parameters were observed in exposed rats or dogs as compared with control animals. Additionally, 10 rats/dose, including a control group handled in a manner similar 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 compared with control rats. Therefore, the NOAEL is 810 ppm, and no LOAEL is established.

Tátrai and Ungváry (1980) exposed groups of 30 male, CFY rats to 0 or 3500 ppm o-xylene (purity not stated) for 8 hours/day for 6 weeks. Despite increased food and water consumption, the body weight gain in the xylene-exposed group was lower than the controls (absolute data were not provided). 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, but measurements of drug metabolizing enzymes were not made. The authors concluded that the

observed changes in organ weight is consistent with adaptational phase of organic chemical exposure and probably reflects induction of enzymes in the liver. Furthermore, they were unable to provide an explanation for the differences in body weight. Given that there was only a single dose studied, no NOAEL could be established, and the LOAEL is based on body weight differences.

To investigate the potential for exposure to xylene to induce hepatotoxicity, Tátrai et al. (1981) exposed male CFY rats to air containing 0 or 1090 ppm (4750 mg/m^3) o-xylene for 8 hours/day, 7 days/week, for 6 or 12 months. The purity of the o-xylene was not provided. 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 (MFO) system (increased cytochrome P-450 and b-5, cytochrome c reductase, alanine hydroxylase, and aminopyrene N-demethylase). Data are presented in graphical form only. Histologic and histochemical examination of the organs including the liver did not reveal any abnormalities. Examination of the liver revealed moderate proliferation of the smooth endoplasmic reticulum. The hepatic effects are not considered adverse. The LOAEL is 1090 ppm based on decreased body weight gain and a NOAEL could not be established.

Ungváry (1990) exposed groups of male CFY rats to air containing 0, 140, 350, or 920 ppm (600, 1500, or 4000 mg/m^3) xylenes (10% o-xylene; 50% m-xylene; 20% p-xylene; 20% ethylbenzene) for 8 hours/day, 7 days/week for 6 weeks, and then for 5 days/week for 6 months. No differences in body weights were observed in any of the exposed groups as compared with the controls. Treatment-related hepatic effects included the following: increased relative liver weights; hypertrophy of the centrilobular zone of the liver; increased nuclear volume of hepatocytes; proliferation of smooth endoplasmic reticulum; increases in the concentrations of cytochromes P-450 and b_5; and increases in the activities of NADPH, cytochrome c-reductase, alanine p-hydroxylase, succinate dehydrogenase and aminopyrine N-demethylase; decreased hexobarbital sleeping time; and transient increase in glycogen. In general, maximal hepatic effects were achieved by 6 weeks of exposure, and returned to control levels after a 4-week solvent-free period following the 6-month exposure. In further experiments by Ungváry (1990), continuous inhalation exposure of CFY rats to 350, 460, or 1150 ppm (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/day for 6 weeks resulted in similar hepatic effects as 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. Because the authors did not consider the hepatic effects to be adverse, the NOAEL is 920 ppm.

Jenkins et al. (1970) conducted inhalation studies on 12 Sprague-Dawley or Long Evans derived 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 8 hours/day, 5 days/week for a total of 30 exposures over a 6-week period while 14 rats, 15 guinea pigs, 2 dogs,

and 3 monkeys were exposed to 78 ppm o-xylene continuously for 90-127 days; and 14 rats, 15 guinea pigs, 10 dogs, and 12 monkeys were exposed continuously for 90-127 days to control air. 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.

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/day).

4.2.2. Chronic Studies and Cancer Assays

4.2.2.1. Oral studies

Chronic oral toxicity studies are available in rats and mice. The only effects noted in rats are slightly lower decreases in body weight gain and increased mortality largely attributed to gavaging in males, while male and female mice only exhibited hyperactivity following dosing. These studies are described below.

In a National Toxicology Program (NTP, 1986) chronic toxicity and carcinogenesis bioassay, 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, 17.0% ethylbenzene, and 9.1% o-xylene) 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/week for 103 weeks. Hematology and clinical chemistry analyses were not conducted. All animals that died or were killed at study termination were given gross necropsy and comprehensive histologic examinations.

In rats, effects of exposure were limited to high-dose males. Mean body weights were 5%-8% lower in high-dose male rats than controls from week 59 to week 97, with body weights at 103 weeks being 4% less in high-dose males than controls (statistical significance not reported). Survival rates after 103 weeks appeared to exhibit a dose-related decrease (36/50, 25/50, and 20/50 for the control, low-, and high-dose males, respectively), with the decrease being significant ($p=0.04$) in the high-dose males. Although a number of the deaths were attributed to gavage error (3/50, 8/50, and 11/50, respectively), the NTP report (1986) suggested that the dosed males resisted gavaging because of the treatment with xylenes resulting in gavage trauma. The authors did not record observations of the rats' behavior during dosing to reach a definitive conclusion. Therefore, the role of xylene and the mechanism of action cannot be definitively identified as the causal agent. Based on increased mortality, a tentative LOAEL is

500 mg/kg/day with a NOAEL of 250 mg/kg/day; however, this should be taken in the context of possible gavaging errors.

In mice, the only treatment-related effect observed was hyperactivity that 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.

4.2.2.2. Inhalation studies

No chronic inhalation studies are available.

4.2.3. Cancer Studies

4.2.3.1. Oral studies

There are two chronic animal studies that assessed the potential for cancer in animals from exposure to xylenes. However, the studies reach different conclusions. NTP (1986) conducted a study on both sexes of rats and mice. A histopathologic examination of the rats revealed an increased incidence of interstitial cell tumors in the testes of high-dose male animals following survival-adjusted analysis, but this increase is believed to be the result of the incidence recorded in high-dose animals dying between weeks 62-92. The overall incidence of interstitial cell tumors between groups was comparable (43/50, 38/50, and 41/49 for the control, low-dose, and high-dose groups, respectively). Therefore, the marginal increase in this tumor is not ascribed to treatment. The NTP (1986) reported no significant nonneoplastic or neoplastic effects in male or female mice.

Maltoni et al. (1983, 1985) exposed groups of 40 male and 40 female Sprague-Dawley rats to 0 or 500 mg xylenes/kg BW (mix of o-, p-, and m-xylenes; proportion of each isomer not stated) in olive oil orally by gavage 4-5 days/week for 104 weeks, followed by discontinuation of dosing to study termination at 141 weeks. Although Maltoni et al. reported an increase in the overall number of malignant tumors in both treated males (14/40 vs. 11/50 for controls) and females (22/40 vs. 10/50 for controls), further study data, such as survival rates and/or specific tumor types, were not provided. The study noted that the time to first tumor was 33 weeks. The decision to report the incidence of animals with tumors and not describe the types of tumors and target organ increases the uncertainty associated with the study. The use of a single high dose raises questions about the ability to extrapolate to a low dose.

4.2.3.2. Inhalation studies

No cancer studies by inhalation were found

4.3. REPRODUCTION AND DEVELOPMENTAL STUDIES

4.3.1. Reproductive Studies

4.3.1.1. Oral Studies

No studies were found in the searched literature regarding the potential for ingested xylene to affect reproduction in animals.

4.3.1.2. Inhalation

In a one-generation reproduction study, 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/day, 5 days/week, for 131 days prior to mating, with exposure continued in the females during GD 1-20 and lactation days 5-20 (Bio/dynamics Inc., 1983). Two additional 500 ppm groups were included: only the males were exposed in Group V, and only the females were exposed in Group VI. Potential pup exposure to xylenes was only through milk.

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 killed after the mating period for gross post mortem examination; the remaining half were killed and examined 21 days later. One-half of the Group I F_0 females and Group IV F_0 females were killed on gestation day 21 for developmental toxicity evaluation: the results of this evaluation are found 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 killed 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 killed on day 49. Randomly selected pups from each group (one/sex/litter) were given a complete gross post mortem 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 as compared with control males. Although the female mating index in Group III and Group VI was significantly lower than controls (85 and 85%, 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. The NOAEL is 500 ppm.

4.3.2. Developmental Studies

4.3.2.1. Oral studies

Pregnant CD1 mice were administered mixed xylenes (60.2% m-xylene, 9.1% o-xylene, 13.6% p-xylene, 17% ethyl benzene) by gavage in cottonseed oil three times daily at dosages of 0, 520, 1030, 2060, 2580, 3100, or 4130 mg/kg/day during gestation days (GD) 6-15 (Marks et al., 1982). Mice were killed on GD 18 and maternal and fetal endpoints were assessed. The highest dose was lethal to 15/15 dams, while the 3100 mg/kg/day dose killed 12/38 dams and resulted in decreased body weight gain (49% of controls for GD 1-18; $p<0.05$). Fetal body weights were significantly decreased in fetuses from dams treated with 2060 mg/kg/day and greater (88, 80, and 72% of controls, respectively; significant trend $p< 0.05$), while increased resorptions occurred only at 3100 mg/kg/day. A significant increase ($p<0.01$) in the percent of fetuses with malformations, consisting primarily of cleft palate, was observed in the groups treated with 2060 mg/kg/day or greater (3.4, 7.8, and 9.1% of controls, respectively). Litter incidences were not provided. No maternal or developmental effects were reported for the 520 or 1030 mg/kg/day groups. The LOAEL for maternal toxicity is 3100 mg/kg/day based on mortality and decreased body weight gain, and the NOAEL is 2580 mg/kg/day. A tentative developmental toxicity LOAEL is 2060 mg/kg/day based on decreased fetal body weights with a NOAEL of 1030 mg/kg/day.

Nawrot and Staples (1980) reported the results of a study in which pregnant CD-1 mice were administered m-, o-, or p-xylene by gavage at approximate dosages of 780, 1960, or 2610 mg/kg/day (0.30, 0.75, or 1.00 ml/kg BW) during GD 6-15, or at a dose of 2610 mg/kg/day during GD 12-15. Exposure to the mid- and high-dose of o- or p-xylene and the high-dose of m-xylene during GD 6-15 resulted in overt maternal toxicity and a significantly increased incidence of resorptions. The mid- and high-dose o- or p- xylene exposed groups (GD 6-15) additionally had an increased incidence of cleft palate. Exposure to 2610 mg/kg/day of any isomer during GD 12-15 resulted in a significant increase in maternal lethality, with exposure to p- or m-xylene additionally resulting in significantly increased incidences of fetal malformations, particularly cleft palate. Subsequent studies on the developmental effects of m-xylene were conducted: mice were administered m-xylene at doses of 1960 or 2610 mg/kg/day during GD 12-15 or GD 6-15. While exposure to m-xylene during GD 12-15 did not result in overt toxicity at the low-dose and did not significantly increase the incidence of malformations, exposure during GD 6-15 did result in a low but statistically significant increase in the incidence of cleft palate in the high-dose group in the absence of overt maternal toxicity (4.4% vs. 0.0% for controls; not stated if fetal or litter incidence). The authors considered this increase to be low and characterized m-xylene as a weak teratogen. Further details were not provided.

4.3.2.2. Inhalation studies

A number of developmental toxicity studies are available although the results are equivocal. One study did not identify a maternal or developmental toxicity LOAEL at the concentrations tested. Another series of studies identified an effect on postnatal development only in female offspring as assessed by the Morris water maze test; no other effects were observed. Although retarded development of fetuses from exposed dams was evident in several studies as indicated by incomplete or non-ossification of skeletal structures and/or decreased fetal body weights, these studies are of limited use because the data were reported as fetal incidences instead of litter incidences, and generally lacked the reporting of other key data. Overall, an adequate assessment of the data was not possible.

No maternal or developmental effects were observed following exposure 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/day on GD 6-15 (Litton Bionetics, 1978a). The NOAEL is 400 ppm.

In a one-generation reproduction study described in Section 4.3.1.2, one-half of the Group I F_0 females (20 females; control group) and Group IV F_0 females (12 females; 500 ppm mixed xylenes by inhalation for 6 hr/day, 5 d/week, for 131 days prior to mating and during GD 1-20) were killed on GD 21 for developmental toxicity evaluation (Bio/dynamics Inc., 1983). 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 post mortem examination. Corrected terminal body weights (corrected for gravid uterine weights) of exposed females were statistically increased as compared with 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 compared with controls (110% of controls), kidney weights relative to body weights were comparable to 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 and control groups for mean number of corpora lutea, implantations, live fetuses, mean percentage of live fetuses/implants, or fetal sex ratios. Although the high-exposure 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. There were no dams with whole litter resorption. No definitive treatment-related external, visceral, or skeletal malformations/variations were observed. The report stated that high-dose fetuses 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 decreased in female fetuses from Group IV (93% of controls); however, male fetal weights were comparable to controls. This marginal decrease in body weight only in female pups is difficult to assess due to the small sample size: only 12 litters from exposed dams were evaluated compared with 20 for controls.

The maternal toxicity NOAEL is 500 ppm, and the developmental toxicity LOAEL/NOAEL is not established.

Mean pup weights were statistically decreased in Groups II, III, and IV on lactation day 4 (post-pooling) as compared with controls. The decreased weights were most likely not the effect of treatment, but rather 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/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 decreased mean pup weights on lactation day 21 (90% of controls) and statistically decreased terminal body weights at 49 days of age (as % of controls: males: 92%; females: 93%). However, despite the marginal decreases observed in mean pup weights in Group IV, no marginal decreases in body weights were observed in pups from Group VI, in which dams were exposed to the same concentration of xylene for the same period of time as dams in Group IV. Therefore, 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-concentration groups (Groups III and IV) also had statistically 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. The systemic and reproductive toxicity NOAEL is 500 ppm.

To evaluate the effects of prenatal exposure on postnatal 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% ethyl benzene) by inhalation for 6 hour/day on GD 7-20 and allowed to litter. Litter size was not standardized, but litters with less than six pups were not used. From each litter, two males and two females were kept for behavioral testing, 1 male and 1 female were kept in standardized housing and left undisturbed other than feeding and taking body weight measurements until 3 months when they were tested in the Morris water maze test, and 1 male and 1 female were kept in enriched housing (cages contained various toys) and tested for rotarod, open field, and Morris maze performance at about 3 months of age.

Exposure to xylene did not affect maternal clinical signs, body weight gain, or food consumption. Control and exposed groups also had a similar gestation period, number of pups/litter, and sex distribution/litter. The number of litters available for evaluation in the control and exposed groups was 13 and 15 litters, 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. Absolute brain weights were significantly decreased on postnatal day (PND) 28 when the data were combined for males and females, but 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 significantly delayed by one day in exposed litters due to the ability of only 4 pups to right themselves. No differences were observed in open field, and

the decreased rotarod performance in exposed female pups reported by the study authors was not statistically significant. Offspring from xylene-exposed rats that were raised in the enriched environment showed no difference in the Morris maze test compared with controls. Offspring from exposed rats that were raised in the standard housing, however, had impaired performance. Testing at 12 weeks showed a nonsignificant trend ($p=0.059$) for increased latency for finding the platform in the beginning of the learning test. At 16 weeks, they used significantly more time to find a platform hidden in the center of the pool. Further analysis revealed the effect was limited to the female offspring, and that these females had an increase in the swimming length, while swim speed was unaffected.

In a study designed to investigate the persistence of the decreased Morris water maze test performance of the offspring from the xylene-exposed (Mol:WIST) female rats, the female offspring raised in the standard housing were continued on the study and 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 from exposed rats only during the first trial of a testing block, while the next two trials resulted in similar latencies between exposed and control rats. The increased latency again corresponded with increased swimming length. No other 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. studies (1995; 1997) found that prenatal exposure to xylenes affected the performance of female offspring in the Morris water maze test: it took the female offspring longer to find the platform. While swim length was lengthened, swim speed was unaffected, indicating a cognitive rather than motor effect. This study is limited, however, in that a dose-response is lacking because only one concentration was tested. Additionally, no clear effect was observed in any of the other neurological tests.

Hass and Jakobsen (1993) exposed groups of 36 pregnant, female Wistar rats to air containing 0 or 200 ppm technical xylene (composition not provided) for 6 hours/day during GD 6-20. On GD 21, two-thirds of the rats were killed and were used to assess developmental toxicity. One-third of the rats were allowed to litter. Developmental milestones and rotarod performance were assessed in 8 offspring (4 males and 4 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 vs. 2/22 control litters. In the postnatal study, statistically decreased rotarod performance was observed in female pups on postnatal days 22 and 23, and in male pups on postnatal day 23. This study is limited in that only one exposure concentration was tested and only a limited battery of behavioral tests were used. Additionally, Hass et al. (1995) state that the testers were not blind to the exposure status of the animals.

Rosen et al. (1986) exposed 18 to 21 pregnant Sprague-Dawley rats to 800 or 1600 ppm p-xylene (3500 or 7000 mg/m^3; 99% pure) on GD 7-16. The treatment did not affect litter size or weight of pups at birth or on post natal day (PND) 3; central nervous system development as measured by the acoustic startle response on PND 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 significant decrease in maternal body weight gain in the 1600 ppm dams (74% of controls). The maternal toxicity LOAEL is therefore 1600 ppm based on decreased body weight gain and the NOAEL is 800 ppm. The developmental neurotoxicity NOAEL is1600 ppm.

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/day during GD 9-14, and were killed on GD 21. No differences in maternal body weights, fetal deaths, mean fetal or placental weights, or external or visceral malformations were noted in the exposed group compared with the controls. Although the study authors reported an increased frequency of fused sternebrae and extra ribs in the fetuses from the exposed group as compared with the controls, the frequency was based on the number of affected fetuses rather than the affected litters.

In a subsequent study, Ungváry and Tátrai, (1985) exposed groups of pregnant CFY rats (19-23) to 0, 60, 440, or 780 ppm (250, 1900, 3400 mg/m^3) xylenes (composition not specified) for 24 hours/day during GD 7-15. The animals were killed on GD 21. Groups of pregnant CFLP mice (17-18) and pregnant NZ white rabbits (10) were exposed to 0, 115 or 230 ppm (500 or 1000 mg/m^3) xylene 3 x 4 hours/day intermittently or to 115 ppm (500 mg/m^3) o-xylene, m-xylene, or p-xylene on GD 6-15 or 7-20, respectively. The mice and rabbits were killed on GD 18 and 30, respectively. Although an increase in the percentage of skeletal retardation (not further described) in rat and mouse fetuses was reported, the increase was not related to concentration and the litter incidence was not given. Rats exposed to 780 ppm xylene additionally had an increased percentage of dead or resorbed fetuses (13% vs 5% for controls). In rabbits, exposure to 230 ppm xylene resulted in 3 maternal deaths, an increased relative liver weight in the dams, and an increased number of abortions. The rats exposed to all three concentrations of xylene each had about 30% incidence of skeletal abnormalities while the high concentration had a higher percentage of dead or reabsorbed fetuses and an average of 13% weight reduction. The mice had a slight increase in skeletal abnormalities and fetal weight decrement at 115 ppm for each of the individual isomers and 230 ppm of mixed xylene, but not for the mixed xylene at 115 ppm. When considering the incidence of all malformations (stated only as skeletal retarded fetuses and minor abnormalities, extra ribs), none of the treatments differed significantly from the controls for the mice or rat models. Of the pregnant rabbits exposed to 115 ppm of the individual isomers or mixed xylenes the only effect seen was a slight decrement in fetal weight in the female offspring. In all cases the data are reported as fetal incidence and not litter incidence. Furthermore, the nature of the skeletal effects is not further described. Other parameters measured did not differ significantly from controls. The authors conclude that based on these studies and others cited in their report, xylenes are, at most, only slightly developmentally toxic. Given the manner in which the data are reported neither maternal nor developmental effect levels can be determined.

To investigate the effect of xylene inhalation on the liver of pregnant and nonpregnant rats and pups of exposed litters, pregnant Wistar rats were exposed to 2600 ppm xylenes (11,284 mg/m^3) (purity and composition not stated) for 8 hours/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 if nonpregnant controls were also included) (Kükner et al., 1997/98). Biochemical analysis of the livers from pregnant rats exposed to xylene revealed minimal increases in aspartate aminotransferase (AST; 18%;), alanine aminotransferase (ALT; 19%;), alkaline phosphatase (ALP; 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, 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 from exposed pregnant or nonpregnant rats, or from fetuses from exposed litters.

To address potential differences between the 3 xylene isomers abilities to cause maternal or developmental toxicity, groups of 15-30 pregnant, CFY rats were exposed by inhalation to air containing measured concentrations of 35, 350, or 700 ppm (150, 1500 or 3000 mg/m^3) of o-, m-, or p-xylene (analytical purity; actual purity not provided) continuously during GD 7-14 (Ungváry et al., 1980). 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 (GD 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 GD 0-21; $p<0.05$). Dams exposed to 350 or 700 ppm o-xylene had statistically but slightly elevated 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. From the way that the data are reported it is not known if these effects are statistically and biologically relevant.

Exposure to 700 ppm m-xylene resulted in a decreased number of mean implantations/dam, while 700 ppm p-xylene resulted in increased postimplantation loss and a corresponding decreased litter size. Fetal body weights were statistically decreased ($p<0.01$ or 0.05) 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 histopathogic or electron microscopic evaluation of organs in fetuses from exposed dams. Although fetuses were examined for external, visceral, and skeletal anomalies, a proper evaluation of the data could not be made because litter incidence rates were

not reported (only fetal incidence rates were provided). The authors report a statistical increase in the fetal incidence of an extra rib in the 700 ppm — and p-xylene groups, and a statistically increased incidence of skeletal retardation in the 700 ppm o-xylene group and in all p-xylene exposed groups.

Mirkova et al. (1983) exposed groups of pregnant female 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/day, 5 days/week during GD 1-21. On GD 21, a number of the animals were killed 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 study authors reported numerous manifestations of toxicity in mid- and high-concentration groups, including a statistically increased percentage of post implantation loss/implantations (10.7% and 14.9%, respectively, vs. 5.5% for controls); statistically decreased fetal body weights (3.20 g and 3.17 g, respectively, vs. 3.64 for controls) and statistically increased percentage of hemorrhages in fetuses (46% and 53%, respectively, vs. 31% for controls). Although the authors additionally 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 statistical decrease in pup weight on post natal days 7 and 21 was also reported for the mid- and high-concentration groups, but data were again not provided. The data from this study are limited by numerous factors, including: composition and purity of xylenes not provided, incomplete description of methods, inadequate litter size for proper fetal evaluations, high incidence of fetal hemorrhages in the control group (suggesting the health of the animals is questionable), and incomplete reporting of results. Therefore, neither maternal nor developmental toxicity LOAEL/NOAEL are established.

4.4 OTHER STUDIES

4.4.1 Neurotoxicity Studies

4.4.1.1 Prechronic oral studies

Groups of 10 male and 10 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 17.0% ethylbenzene, and 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 (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. 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% less than controls, respectively). No treatment-related gross or microscopic changes 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 neurological effects, and the NOAEL is 1000 mg/kg/day.

Central nervous system toxicity was also observed in rats and mice following acute oral administration of mixed xylenes in several prechronic studies (NTP, 1986). Groups of 5 male or 5 female B6C3F1 mice or F344/N rats were administered a single dose of 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, groups of 5 male or female F344 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 as compared with controls, while females dosed with 125 or 1000 mg/kg had body weight gains 17% and 26% lower than controls.

4.4.1.2 Prechronic inhalation studies

Korsak et al. (1992) exposed groups of 12 male, Wistar rats to toluene, m-xylene, or their 1:1 mixture for 6 hours/day, 5 days/week at a concentration of 100 ppm for 6 months or 1000 ppm for 3 months. The study authors employed the rotarod test 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 the ability of the animals to remain on the rod for a period of 2 minutes. The animals are trained to perform the task, exposed to chemical or control gas, and evaluated at defined intervals. Rats exposed to m-xylene alone exhibited significantly decreased rotarod performance and decreased spontaneous activity as measured 24 hours after termination of the exposures when compared to controls. The data are presented only in graphical form which limits the ability to derive accurate values. The percentage of failures in the rotarod test was roughly 60% in rats exposed to 1000 ppm for 3 months, and 35% in rats exposed to 100 ppm for 6 months, compared with 0% for controls. The spontaneous motor activity in rats exposed to 100 ppm for 6 months was roughly half that of controls: approximately 400 movements/hour vs. approximately 800 movements/hour for controls (graph not provided for rats exposed to 1000 ppm m-xylene for 3 months). No exposure-related changes in body weight, absolute or relative organ weights; or clinical chemistry or hematology parameters were noted. The LOAEL is 100 ppm based on decreased rotarod performance and spontaneous activity, and the NOAEL is not identified.

In a second study, Korsak et al. (1994) exposed groups of 12 male Wistar rats to 50 or 100 ppm m-xylene or n-butyl alcohol, or their 1:1 mixture at 50:50 ppm or 100:100 ppm for 6 hours/day, 5 days/week for 3 months (purity of chemicals not provided). Exposure to 50 or 100 ppm m-xylene alone resulted in decreased rotarod performance starting at 1 month of exposure and remaining at the same level until the end of the 3 month exposure, with the decreases being statistically significant in the 100 ppm exposure group. Because the results are presented in graphical form, the actual numerical data are not provided. The percentage of failures was roughly 8% and 33% for the 50 and 100 ppm groups, respectively, vs. 0% for the controls. Rats exposed to 50 or 100 ppm m-xylene alone also had statistically increased sensitivity to pain at the end of the 3 month exposure as determined by the hot plate behavior test (latency of the paw lick response was 8.7 and 8.6 seconds, respectively, vs. 12.2 seconds for the controls). No exposure-related changes in body weight gain; absolute or relative organ weights; hematology parameters; or in hepatic microsomal monooxygenases, lipid peroxidation, or triglyceride levels were noted. Although a statistically increased sensitivity to pain was noted as measured by the hot plate behavior test in the xylene-exposed groups, the response did not appear to be related to concentration. Based on these data alone, it was not clear that the decreased latency of the paw lick response observed in the 50 and 100 ppm m-xylene groups was definitively an effect of m-xylene exposure. Therefore, the LOAEL is 100 ppm based on decreased rotarod performance, and the NOAEL is 50 ppm.

Pryor et al. (1987) conducted studies to examine the potential for xylene to cause ototoxicity. In the study groups of twelve weanling, male F344 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/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 frequency of the tones tested were 4, 8, 12, and 20 kHz, with the sound levels (decibels) varying depending on the frequency. Results were presented only in graphical form, with actual data not provided. All xylene-exposed rats had concentration-dependent increases in the behavioral auditory thresholds and BAER thresholds relative to the 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. Based on increased behavioral auditory and BAER thresholds, the LOAEL is 800 ppm and the NOAEL is not determined.

Groups of male Sprague-Dawley rats were exposed by inhalation of air containing 1000 ppm xylene (o-xylene: 1.5%; m-xylene: 65%; p-xylene: 32%; ethylbenzene: 2.5%), 1000 ppm n-hexane, or 1000 ppm of n-hexane mixed with xylene, for 18 hours/day, 7 days/week for 61 days (Nylén and Hagman, 1994). Two days following exposure, rats exposed to xylene alone had statistically decreased body weights and a slight loss in auditory sensitivity as recorded by

auditory brainstem response as 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., one of the two dogs exposed to 780 ppm o-xylene for 8 hours/day, 5 days/week for 6 weeks exhibited tremors throughout the exposure (Jenkins et al., 1970). No additional information is provided.

To evaluate whether xylene exposure results in accelerated aging of the central nervous system, Gralewicz et al. (1995) exposed eight-month-old, male LOD-Wist rats (20 per dose level) to air containing 0, 100, or 1000 ppm "pure" m-xylene (exact purity not provided) for 6 hours/day, 5 days/week, for 3 months. The effects of xylene exposure on brain aging were evaluated by measuring spontaneous neocortical spike and wave discharges (SWD) activity with electroencephalograms and assessing spatial learning in an 8-arm radial maze. As animals age, SWD activity is supposed to increase, while spatial learning abilities decrease. Unlike the controls, rats exposed to 100 or 1000 ppm m-xylene did not exhibit a shortening of the duration needed to complete a trial in the radial maze with successive days (response speed), and did not exhibit a consistent decrease in omission errors (number of arms in the maze omitted during a 5-minute period when rats allowed to explore the maze). However, mid- and high-concentration rats exhibited retarded age-related SWD activity as compared with controls. Therefore, although central nervous system effects were noted in rats exposed to m-xylene, the results were not consistent with just accelerated CNS aging. The LOAEL is 100 ppm, the lowest dose to which the animals were exposed, based on differences in radial maze performance and spike and wave discharge activities, and a NOAEL is not identified.

In a study conducted by Savolainen et al. (1979a) 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/day, 5 days/week, for 5 to 18 weeks, with or without concomitant exposure to ethanol in the drinking water. Exposure to xylene alone resulted in an increase in the microsomal superoxide dismutase activity in the brain at the end of the exposure, and transient, decreased preening frequency.

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

Studies have also been conducted assessing 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.

de Gandarias et al. (1995) administered 1.6 ml/kg/day reagent grade xylene (isomer(s) not specified) via the intraperitoneal route to 3 month old Sprague-Dawley rats for three consecutive days while the control group received 0.9% sodium chloride solution. Following the third administration, the rats were killed and the brains were removed, fixed, and stained for met-enkephalin with polyclonal antisera. The researchers examined the corpus callosum, parietal cortex, caudatus-putamen, olfactory tubercule, hypothalamic medial preoptic area, globus pallidus, and central nuclei of the amygdala. The data revealed a reduction in immunostaining for met-enkephalin with respect to corpus callosum when compared with controls, in the medial preoptic area of the hypothalamus and the globus pallidus and a trend for lower levels in the olfactory tubercule. The authors conclude that the data demonstrate a role for enkephalins in the neurotoxic mechanism of action.

In a similar study, de Gandarias et al (1993) administered 1.6 ml/kg/day regent grade xylene (isomer(s) not specified) via the interperitoneal route to 3 month old Sprague-Dawley rats for three consecutive days. The control group received a similar amount of saline solution (0.9% sodium chloride solution). Following the third administration, the rats were killed and the brains removed, sectioned, and homogenized. The brains were sectioned to isolate the frontal, parietal and occipital cortices, olfactory bulb, thalamus, hypothalamus, pituitary gland, striatum, amygdala, hippocampus, cerebellum, medulla, and pineal gland. Assays on aminopeptidases, enzymes that are associated with the activity of the neurotransmitters, were measured for each of the sections noted above. The study found a significant decrease in the activity of leucine-aminopeptidase in the thalamus, but not in other sections of the brain or in changes in lysine-aminopeptidases in the thalamus or other sections of the brain.

Intraperitoneal injection constitutes a nonregular method of administration and therefore these studies are not useable for the derivation of dose response. However, these studies provide additional information on neurological effects arising from exposure to xylenes.

4.4.2 Genotoxicity

The genotoxicity of commercial xylene and all 3 individual isomers has been extensively tested and the results are, for the most part, negative. All studies evaluated by the GENTOX panel and cited in the GENTOX database are negative except for one study for which no conclusion was drawn (GENTOX, 1999). Xylene is not mutagenic in bacterial test systems with *Salmonella typhimerium* (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). Xylene also does 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 xylene, but not o- and m-xylene, is weakly mutagenic in *Drosophila* recessive lethal tests (Donner et al., 1980). No increase in the frequency of sister chromatid exchanges were observed in peripheral lymphocytes from individuals exposed to

xylene 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

Only a limited number of studies were found in the searched literature comparing the toxicity of the individual xylene isomers. Although differences in toxicity are seen in some studies among the isomers, no consistent, significant differences in the potency of the isomers following oral or inhalation exposure have been identified.

Condie et al. (1988) did not find any significant differences in the toxicity of the individual isomers in an experiment in which 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 and deaths were attributed to treatment. Male rats receiving 2000 mg/kg/day of each isomer had statistically lower body weights (88-94% of controls), while the 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 128-148% of controls). Certain treatment groups also had decreased spleen or thymus weights. No treatment-related effects were observed in hematology, clinical chemistry, or urinalysis parameters. 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 was 1400 ppm for all isomers, with an EC_{50} (concentration producing half-maximal decreases in response rate) of 6176, 5179, or 5611 ppm for m-xylene, o-xylene, and p-xylene, respectively. The operant response was biphasic, with concentrations of 1400 to 2400 ppm producing increased rates of response, and a concentration of 7000 ppm suppressing the response rate and also producing gross ataxia and prostration. The minimally effective concentrations for the inverted screen test are 3000 ppm for — and o-xylene, and 2000 ppm for p-xylene, while the EC_{50} values for performance on the inverted screen test are 3790, 3640, and 2676 ppm for m-xylene, o-xylene, and p-xylene, respectively. Motor ability was recovered approximately 5 to 15 minutes after exposure. The study authors concluded that there was no consistent, significant difference in the potency of the individual isomers. While o-xylene exhibited a more potent effect on operant behavior, p-xylene more severely affected motor performance.

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 concentration-related increase in group motility, while 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 ten male Wistar rats were exposed to approximately 3000 ppm o-, m-, or p-xylene for six hours, with rotarod performance measured before and after termination of the exposure. The results of the testing given in terms of the number of failures/number of tested animals was as follows: o-xylene at average concentration of 3027 ppm was 19/20; m-xylene at average concentration of 3093 ppm was 6/20; p-xylene at average concentration of 3065 ppm was 1/20.

To address the potential for the 3 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 GD 7-14. Dams were sacrificed on GD 21. For a complete description of this study, the reader is referred to Section 4.3.2.2. Unfortunately, the usefulness of this study is limited because much of the actual data were not provided and the analyses of developmental toxicity was based on fetuses as the experimental unit instead of litters. The general conclusion is that exposure to m-xylene is the most toxic to the dams, while fetal toxicity varied with the isomer; for example, m-xylene exposure resulted in decreased number of mean implantations/dam, p-xylene exposure resulted in increased post implantation loss and corresponding decreased litter size, and all concentrations levels of p-xylene and the highest concentration of o-xylene resulted in increased fetal incidence of skeletal retardation.

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 MAC of o-, m-, and p-xylene was 0.00118 ± 0.00009, 0.00139 ± 0.00010, and 00.00151 ± 0.0007 atm, respectively, with a difference of MAC values of less than 30% among the isomers.

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

Technical grade mixed xylenes, the form most commonly used as a commercial solvent, is a blend of three isomers of dimethylbenzene (o-, m-, and p-xylene) and frequently contains a significant portion of ethylbenzene. Mixed xylenes, rather than individual isomers, is the composition to which the public is most likely exposed, 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) vary considerably depending on its source.

The most pronounced and consistent effect of exposure to xylene in humans and animals is central nervous system disturbance. Central nervous system effects in humans following

inhalation exposure to xylene include headache, vertigo, nausea, fatigue, irritability, dizziness, impaired concentration, or confusion. Studies with controlled inhalation exposures in males have yielded mixed results following neurobehavioral testing. A number of studies have found that xylene exposure to p- or m-xylene concentrations ranging from 70-400 ppm for up to 4 hours either did not affect the performance of subjects in neurobehavioral testing (Olsen et al., 1985) or actually improved performance (Laine et al., 1993; Savolainen et al., 1981;1985b).

Other studies have found a correlation between acute exposure to m-xylene at concentrations ranging from 64-400 ppm for up to 4 hours and impaired performance (Savolainen et al., 1979b; 1980; 1984; 1985a; Savolainen and Linnavo, 1979; Savolainen and Riihimäki, 1981; Dudek et al., 1990; Gamberale et al., 1978; Seppäläinen et al., 1983; 1989; 1991). Some of the studies evaluating the effects of repeated exposure to m-xylene indicate the development of tolerance in exposed subjects (Savolainen et al., 1980; Savolainen and Riihimäki, 1981). Other studies demonstrate that workers exposed to low levels of xylenes experience subjective symptoms while workers exposed to moderate levels of xylenes in occupational settings experience sensations consistent with narcotic effects (Uchida et al., 1993). Case reports of individuals exposed to high concentrations of xylenes by inhalation, ingestion, or intravenous injection report severe respiratory effects including respiratory failure (Morley et al., 1970; Abu Al Ragheb et al., 1986; Recchia et al., 1985; Sevcki et al., 1992). The respiratory effects are consistent with depression of the respiratory center of the brain.

Animal studies reporting nervous system disturbances following acute, subchronic, or chronic via oral exposure to xylenes include the NTP (1986) study and the Wolfe studies (1988a; b). The NTP study (1986) reports transient lethargy, unsteadiness, tremors, and paresis occurring in mice dosed with 2000 mg/kg/day for 13 weeks and transient hyperactivity was noted in mice dosed with 1000 mg/kg/day for 2 years. Central nervous system effects observed in rats or mice following acute or subacute oral dosing with xylenes are consistent with the narcotic and anesthetic effects of the chemical, and include tremors, prostration, slowed breathing, lack of coordination, loss of hindleg movement, and hunched posture. The higher mortality observed in male rats orally dosed with 500 mg/kg/day for 2 years is primarily attributed to gavage error, but the possibility that the males resisted gavaging because of treatment could not be excluded. Mortality related to complications from gavaging was also observed in 13-week studies in rats by Wolfe (1988 a; b). Studies designed to investigate xylene-induced nervous system disturbance in animals following repeated exposures were primarily limited to inhalation exposures in rats. Exposure of rats to 100 ppm m-xylene for 3 or 6 months resulted in decreased rotarod performance and/or decreased spontaneous activity (Korsak et al., 1992; 1994). Ototoxicity was noted in rats exposed to 800, 1000, or 1200 ppm xylenes for 14 hours/day for 6 weeks (Pryor et al., 1987) or 1000 ppm xylenes for 18 hours/day for 61 days (Nylén and Hagman, 1994). Differences in radial maze performance and spike and wave discharge activities were noted in rats exposed to 100 or 1000 ppm m-xylene for 6 hours/day, 5 days/week for 3 months (Gralewicz et al., 1995). One study of central nervous system disturbances in dogs reports tremors in one of two dogs exposed to 780 ppm o-xylene for 8 hours/day, 5 days/week, for 6 weeks (Jenkins et al., 1970).

The low molecular weight and lipophilic nature of xylenes (log K_{ow} of 3.12-3.20) allow the solvent to readily cross the blood:brain barrier. Studies investigating the distribution of radiolabeled-xylenes following inhalation exposure confirm high concentrations of xylenes in the brain, and central and peripheral nervous system immediately after exposure, with elimination often occurring by 1-hour post-exposure (Bergman, 1983; Carlsson, 1981; Kumarathasan et al., 1997; Ghantous and Danielsson, 1986). The transient nature of many of the xylene-induced nervous system disturbances in humans and animals is likely attributable to rapid elimination of xylene. The mechanisms whereby xylenes affect the nervous system are not known. An *in vitro* study using human and rat cell membranes demonstrated that xylene and other solvents with anesthetic properties can bind in hydrophobic pockets in integral cell membrane proteins, thereby altering the properties of integral enzymes (Tahti, 1992). Other studies find that xylene exposure affected the enkephalinergic neuromodulatory system (de Gandarias et al., 1995), catecholamine neurotransmission by altering levels of dopamine and noradrenaline (Andersson et al., 1981), and levels of brain acetylcholine and glutamine (Honma et al., 1983). Xylene exposure was also found to decrease transport of cellular materials to axons and nerve ending regions in rats (Padilla and Lyerly, 1989), affect microsomal superoxide dismutase activity in the brain of rats (Savolainen et al., 1979a), and result in findings compatible with astrogliosis in gerbils (increased brain concentrations of glial fibrillary acidic protein, S-100 protein, and DNA) (Rosengren et al., 1986). Xylene exposure does not greatly affect neutral or basic aminopeptidase activities in the brains of rats (de Gandarias et al., 1993).

Data demonstrating that xylene is hepatotoxic are limited. In humans, data are limited to case reports and indicate only transient effects. In one case report, Morley et al. (1970) report an accidental exposure to approximately 10,000 ppm xylene. The autopsy of a worker that died revealed hepatic congestion with swelling and vacuolization of cells in the centrilobular areas. The other two exposed workers that survived had only slight hepatic impairment as indicated by a rise in serum transaminase over 48 hours following the exposure, followed by a return to normal levels.

The nature of the hepatic changes in rats following subchronic oral or inhalation exposure to xylenes is consistent with the induction of enzymes associated with metabolism of chemicals and in most of the reports are not considered to be adverse by the authors. Condie et al. (1988) reported that oral exposure to 750 or 1500 mg/kg/day for 90 days resulted in increased liver weights and a modest increase in serum liver enzymes without abnormal histopathologic findings. Rats fed 10 mg/kg/day o-xylene for up to 6 months developed membrane-bound hepatocellular vacuoles at 1 month, the appearance and size of which did not change over time with continued feeding (Bowers et al., 1982). Following inhalation exposure to o-xylene or mixed xylenes at concentrations of 140 ppm to 3500 ppm for 6 weeks to 1 year, responses in rat liver included, but were not limited to, increased liver weights, hepatocellular hypertrophy, increased smooth endoplasmic reticulum, and induction of enzymes of the hepatic MFO system, all without significant histopathologic and/or histochemical changes (Tatrai and Ungvary, 1980; Ungvary 1990; Tatrai et al., 1981). The hepatic effects reported by Ungvary (1990) were maximal by 6 weeks of exposure, and returned to control levels after a 4-week solvent-free period.

Information regarding the potential for xylene exposure to cause renal toxicity is also limited. No conclusive data addressing xylene-induced renal toxicity in humans are available. One case report indicates only transient renal effects (Morley et al., 1970) and no renal effects are noted in workers chronically exposed to xylenes at low concentrations (Uchida et al., 1993). Condie et al. (1988) report increased kidney weights in rats gavaged with mixed xylenes at dosages of 750 or 1500 mg/kg/day for 90 days. Microscopic evaluation of the kidneys reveal a dose-related increase in hyaline droplet formation in male rats and an early appearance of minimal chronic nephropathy in females. No nephrotoxic effects were observed in rats gavaged with up to 2 g/kg/day for 5 days/week for 4 weeks (Borriston Laboratories, Inc., 1983), or in rats or mice of either sex gavaged with up to 500 mg/kg/day or 1000 mg/kg/day, respectively, for up to 2 years (NTP, 1986). Following inhalation exposure, no histopathologic lesions of the kidney were found by Carpenter et al. (1975), or Jenkins et al. (1970).

Body weight decrements are a systemic effect of xylenes that are frequently reported in animals following oral exposure (Condie et al., 1988; Wolfe, 1988a; 1988b; NTP, 1986; Marks et al., 1982) or inhalation exposure (Tátrai and Ungváry, 1980; Tátrai et al., 1981; Rosen et al., 1986; Ungváry et al., 1980; Nylén and Hagman, 1994). The mechanism for this effect is not known. The only correlate found to human exposures is the symptom of poor appetite reported by Uchida et al. (1993) in workers chronically exposed to low levels of xylenes in the workplace.

Developmental toxicity in animals has been observed at doses or concentrations similar to or exceeding those resulting in maternal toxicity. There are two studies investigating the potential mechanism for xylene-induced developmental toxicity (retardations and lethal effects). In a study investigating the role of maternal sex steroid production and metabolism in p-xylene embryotoxicity, Ungváry et al. (1981) report that exposure of pregnant rats to 690 ppm p-xylene (3000 mg/m^3) on GD 10 or GD 9-10 did not affect maternal ovarian and uterine circulation or ovarian hormone secretion rate as measured on GD 11 as compared with controls. However, exposure to p-xylene for 48 hours (GD 9-10) did result in a statistically significant decrease in the peripheral levels of progesterone and β-estradiol and significantly decreased fetal body weights (actual data not provided). It was proposed that the hepatic enzyme induction by p-xylene was responsible for increased metabolism of the sex hormones, which in turn was responsible for the fetal effects. In another study, Ungváry and Donáth (1984) found that exposure of pregnant rats to approximately 350 ppm p-xylene resulted in hyperinnervation or degeneration of noradrenergic nerves of reproductive organs (uterus, ovaries). They propose that damage to the peripheral noradrenergic nerves can result in altered control of uterine and ovarian blood flow and steroid production, resulting in fetal toxicity.

Limited data are available to adequately evaluate differences in sensitivity among species and between sexes to the toxic effects of xylenes. While some differences have been observed in the metabolism of xylenes in humans and animals, it has been proposed that the differences observed may actually be due to the differences in the size of the doses administered to each: the larger doses received by the animals may saturate the glycine-conjugation pathway (the predominate pathway in humans), resulting in the additional formation of glucuronide conjugates (ATSDR, 1995). The relevance of these differences in metabolism in assessing species

susceptibility to xylene-induced toxicity is questionable, because it does not appear likely that metabolites of xylenes are responsible for any significant portion of the toxic effects thus far described.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under EPA's Risk Assessment Guidelines of 1986 (U.S. EPA, 1986a), xylenes are classified into cancer weight-of-evidence Category D - *not classifiable as to human carcinogenicity*. Under the revised draft Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the carcinogenicity of xylenes *cannot be determined because the existing evidence is composed of conflicting data*. These characterizations are based on the availability of studies that both support and mitigate potential for carcinogenicity from exposure to xylenes. Although epidemiological studies investigating the potential for inhaled xylenes to cause cancer reported increased incidence of tumors these studies are limited by the size of the exposed cohort, concurrent exposures to other chemicals and characterization of the xylenes, and are therefore considered insufficient by the criteria set forth by the U.S. EPA (1999).

In a gavage study, NTP (1986) reports *no evidence of carcinogenicity* for male or female rats given 250 or 500 mg/kg, or for male or female mice given 500 or 1000 mg/kg for 2 years in a well-conducted study. A second rodent study (Maltoni et al., 1983; 1985) found increases in incidence of malignant tumors in male and female rats. The second report fails to provide essential information for a full assessment including tumor types and survival rates of the treated animals. No studies were found in the literature regarding the potential for inhaled xylene to cause cancer in animals. Genotoxicity testing of mixed xylenes and all three individual isomers has consistently yielded negative results.

A concern for carcinogenicity from exposure to xylenes could be made based on the structural similarity to benzene which is classified as *carcinogenic to humans* (U.S. EPA, 2000). For two other chemicals with structural similarities to xylenes IARC (2000) found *inadequate evidence in humans for carcinogenicity of ethylbenzene* and *sufficient evidence for carcinogenicity in experimental animals.* The human determination was based on two separate studies of workers occupationally exposed to ethylbenzene but finding no increased risk of cancer. The animal determination was based on inhalation study in mice and rats. The IARC report states that in mice, ethylbenzene increased the incidence of lung adenomas in males and of liver adenomas in females. In male rats, it increased the incidence of renal tubule adenomas and carcinomas. The overall evaluation is that *ethylbenzene is possibly carcinogenic to humans.* For toluene, IARC (1989) found *inadequate evidence in humans for carcinogenicity* and *evidence suggesting lack of carcinogenicity in experimental animals*. The overall evaluation of toluene by IARC conclude that *toluene is not classifiable as to its carcinogenicity to humans.* The data that support these classifications suggest that alkyl substitution of a benzene ring diminishes the potential for carcinogenicity of chemicals with a benzene backbone relative to the nonsubstituted benzene.

The mechanism of action for carcinogenicity of benzene is proposed to be mediated by the formation of an epoxide which is subsequently converted to catechol and benzoquinones (U.S. EPA, 2000). As such, the mechanism requires two adjacent nonsubstituted carbons in the benzene ring to form the epoxide. An alkyl group on the benzene ring diminishes the potential for this reaction. The presence of two alkyl substitutions would be expected to further reduce the potential for oxidation of adjacent carbons. The substitutions are expected to further reduce the potential for formation of quinones. The metabolism of xylenes involves the oxidation of the methyl group to the toluic acid. None of the studies reviewed to date report the formation of epoxides in the metabolism of xylenes.

The IARC (1999) characterization of the cancer potential for xylenes is *inadequate evidence for carcinogenicity* in humans and animals and characterizes xylenes as *not classifiable as to their carcinogenicity in humans.*

4.7. SUSCEPTIBLE POPULATIONS

No definitive data addressing susceptible populations are available. Differences in sensitivity between males and females when they occur in animal studies are inconsistent.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The RfD derived from the NTP (1986) lifetime rat oral gavage study has been withdrawn. That assessment was based on critical effects from the chronic study. These effects were decrease body weight and mortality[1].

There are no long-term human studies that are suitable for generating a dose-response assessment. Therefore, only animals studies were considered. There are a number of studies by the oral route of administration in which effects are seen in animals. The effects include changes in body weight (NTP, 1986; Condie et al., 1988; Wolfe, 1988a;b; Marks et al., 1982), kidney effects (Condie et al., 1988); and neurological effects (NTP, 1986; de Grandarias, 1993;1995).

Changes in body weight is the most consistent effect reported with animals. In the only chronic study available Fisher 344 rats averaged 5-8% lower body weight gains at 500 mg/kg/day (statistical significance not reported) and found no consistent difference between treated mice

[1]The previous IRIS Summary notes hyperactivity in the rat study following gavaging. However the NTP study reports hyperactivity in the mice study only.

(administered xylene up to 1000 mg/kg/day) and vehicle controls. The effects were most pronounced in the subchronic study compared with the chronic study. Condie et al. (1988) report that final weights of male rats was 94% of controls and females were about the same as controls for animals dosed with 1500 mg/kg/day for 90 days. Wolfe (1988b) reports a trend for slightly lower body weights in animals treated with 800 mg/kg/day p-xylene but at 13-weeks the differences were not statistically significant; however, with m-xylene the results were statistically significant at the 200 and 800 mg/kg/day doses for females and 800 mg/kg/day for females (Wolfe, 1988a). A 13-week gavage study with mixed xylenes in rats resulted in slight decreases in body of male and female rats at doses of 1000 mg/kg/day, and for male and female mice at doses of 2000 mg/kg/day (NTP, 1986). For the most part diminished body weight gain in subchronic studies was not found in conjunction with other overt signs of toxicity. Furthermore, the reason for diminished body weight is not clear. In one study, Wolfe (1988b) found a slight reduction in body weight coincided with higher food consumption. In other studies food consumption was not reported or was found to be lower. In none of the studies was the issue of lower body weights addressed from a toxicological standpoint. Factors that mitigate selection of body weight as the critical effect include the absence in most cases of overt signs of toxicity in conjunction with reduced body weight, the minimal difference in body weight in the chronic study compared with shorter-term studies, and the possibility that the reduced body weight was due to lower palatability of food resulting from administration of a noxious chemical.

Kidney effects are reported by Condie et al. (1988) who characterized the condition as a minimal nephropathy. However, these effects were not found in studies conducted in a similar manner and administering comparable doses of xylenes (Wolfe, 1988a;b; NTP, 1986; Borriston Laboratories, 1983). As a result of conflicting results kidney effects was not selected as the critical effect.

Developmental studies reported increases in skeletal malformations in xylenes-treated offspring (Marks et al., 1982; Nawrot and Staples, 1980). The data in these reports are provided as fetal incidence and not litter incidence: a method which does not allow one to identify whether the effect was limited to a single litter, or consistently observed in fetuses across litters. As such, the data are not reported in a manner that is suitable for generating an RfD. The Nawrot and Staples (1980) omit essential data for an accurate assessment of the effects.

The data base on xylenes supports the selection of neurotoxicity as the critical effect. Studies on the distribution and metabolism of xylenes indicate that following absorption into the body xylenes will selectively partition into those tissues with a high composition of fatty acids including the brain (Kumarathasan et al., 1997;1998; Carlsson, 1981; Bergman, 1983). The data suggest that metabolism of xylenes in the brain is slower compared with other tissues of the body. A number of studies provide evidence that xylenes is a neurotoxic chemical. As discussed below, the 13-week study provides evidence as indicated by the activity of dosed animals that xylenes is neurotoxic. Biochemical and morphological effects were reported by Rosengren et al. (1986) who found that exposure to xylene led to increases in brain concentrations of glial fibrillary acidic protein, S-100 protein and DNA: all indicators of brain injury. de Grandarias

(1993, 1995) found decreases in leucine-aminopeptidases and met-enkephalin, both factors associated with neurological activity.

In humans, neurotoxicity is the effect most commonly reported following exposure to xylenes. Uchida et al. (1993), Hipolito (1980), and Klaucke et al. (1982) report that individuals exposed to xylenes under occupational conditions experienced forgetfulness, inability to concentrate, and diminished appetite, nausea, vertigo, mental confusion, and dizziness. However, these studies are not suitable for generating an RfD.

NTP (1986) conducted toxicology and carcinogenesis studies on mixed xylenes by the oral route of exposure. To ensure accurate administration of the chemical the gavage method was used. The complement of tests involved single dose administration, fourteen day studies, thirteen week studies and two year studies on male and female Fisher 344 rats and B6C3F_1 mice. Chronic studies in mice gavaged to doses of 1,000 mg/kg/day indicated no pathological lesions or other toxicological effects were seen in the studies. The authors observed hyperactivity in all high dose mice of both sexes 5-30 minutes following dosing from the fourth week of the study through week 103; however they provided no explanation as the reason for the response. Therefore, the issue of gastrointestinal distress could not be ruled out. The authors report that rats in the chronic study had a higher incidence of mortality but also a higher incidence of gavage-related deaths. The authors state that the higher mortality could be due to increased resistence from gavaging but no notes were taken of the animals behavior during administration. Evidence of chemical-related effects in the chronic studies is limited. In shorter-term studies there is evidence of toxicological effects. A 13-week study in rats notes that body weight of rats dosed with 1,000 mg/kg/day were 15% and 8% lower than that of the vehicle controls after 13 weeks of exposure but there were no other signs of toxicity or compound-related pathologic lesions. In mice the authors report neurobehavioral effects in the animals with the onset 5-10 minutes following administration and lasting for 15-60 minutes. In addition to providing evidence of neurotoxicity the kinetics of the onset and termination of the effects track the pharmacokinetics of xylene (NTP, 1986). The principal study selected for generation of the RfD was the NTP subchronic study in mice, and the critical effect was neurotoxicity based on behavioral effects.

The number of studies employing oral administration in which neurological effects are reported is limited. The principal study chosen is the prechronic NTP (1986) study in mice in which neurotoxicological effects were reported. Groups of 10 male and 10 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 17.0% ethylbenzene, and 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 (NTP, 1986). Observations were made twice daily, and body weight was recorded once per week. At termination of the study, necropsy was performed on all animals and comprehensive histologic examinations 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% less than controls, respectively). No treatment-related gross or microscopic changes were observed. No adverse effects were reported in mice

dosed with 125, 250, 500, or 1000 mg/kg/day. The LOAEL is therefore 2000 mg/kg/day based on neurological effects, and the NOAEL is 1000 mg/kg/day.

Some studies employing oral gavage administration report increases in mortality of animals (NTP, 1986; Wolfe, 1988a; b). While this is clearly an adverse effect the mechanism accounting for the mortality is not provided. The authors of the NTP (1986) study note that a number of the dosed animals died from gavage error. Mortality in the NTP (1986) study occurred in the chronic study but not in the subchronic component which employed higher doses. Several, but not all, of the deaths were attributed to gavage error. The authors indicate that the rats may have resisted gavaging although they do not report on activity of the animals during gavaging. In both Wolfe studies (1988a;b) necropsy of the dead rats revealed foreign material in the lungs of the animals that died prematurely. The author notes that the animals in the high dose group exhibited "excessive" salivation prior to dosing, but offers no additional information on the behavior of the animals. The author attributes the mortality to vehicle/chemical aspiration and does not consider this effect to be chemically related.

5.1.2. Methods of Analysis

The data were analyzed using the NOAEL/LOAEL approach. Standard uncertainty and modifying factors were applied. The LOAEL is 2000 mg/kg/day and the NOAEL is 10000 mg/kg/day based on neurological effects observed in mice at the high dose. The NOAEL of 1000 mg/kg/day was adjusted for duration of dosing using the following equation:

$$NOAEL^*_{[ADJ]} = (1000 \text{ mg/kg/day}) \times (5 \text{ days}/7 \text{ days}) = 710 \text{ mg/kg/day}$$

While this study indicates that the neurological effects were prominent enough to report there is no information on the number of animals experiencing these effects at the respective dose. As such, the reported data are not amenable to benchmark dose derivation.

5.1.3 Oral Reference Dose Derivation

A reference dose was estimated from the $NOAEL_{[ADJ]}$ of 710 mg/kg/day with an uncertainty factor (UF) of 1000 and a modifying factor (MF) of 1. Uncertainty factors of 10 each were applied for inter- and intraspecies extrapolation, and for database insufficiency.

A full factor of 10 for intraspecies extrapolation is applied to account for sensitive members of the population. A full uncertainty factor of 10 is applied to account for interspecies extrapolation because the RfD is derived from animal studies. The pharmacokinetics of xylenes is similar to that of humans (Bray et al., 1949; Riihimaki and Savolainen, 1980; David et al., 1979). In all cases, xylenes administered to animals or humans indicated rapid uptake, partitioning into neutral fats, oxidation and conjugation with glycine, and excretion. However, direct comparisons were not conducted due limited availability of data on the pharmacokinetics in humans. Therefore, a full 10-fold interspecies uncertainty factor is applied.

An uncertainty factor for database insufficiency of 10 is applied to account for the lack of neurotoxicity, and reproductive and developmental toxicity studies by the oral route. There are studies that are suggestive of heritable effects in rodents but are of limited value given the manner of reporting. Although the critical effect is neurotoxicity, there are no dedicated neurotoxicity assays in the data base. Therefore, the data base would be significantly improved by inclusion of multigenerational and neurotoxicity studies.

An uncertainty factor was not applied to account for a subchronic to chronic extrapolation. The neurological effects that were noted in the subchronic studies were not apparent at the slightly lower doses used in the chronic studies. Furthermore, the xylenes does not produce toxic metabolites nor does it bioconcentrate in any of the tissues investigated. This may reflect the metabolism and excretion of xylenes following ingestion. Therefore, it is not anticipated that extended exposure will lead to more pronounced effects. The RfD is based on a NOAEL and so an uncertainty factor to account for a LOAEL to NOAEL is deemed unnecessary.

$$RfD = NOAEL_{[ADJ]} \div UF*MF$$

$$RfD = 710 \text{ mg/kg/day} \div 1000 = 0.7\text{mg/kg/day}$$

This value replaces a previous RfD of 2 mg/kg/day based on increased mortality that was seen with the chronic exposure component of the same study (U.S. EPA, 1987). The RfD includes an uncertainty factor of 10 for intraspecies variability to account for more sensitive members of the population, and interspecies variability to account for extrapolation from rodents to humans. The data indicate that humans, rats, and mice are sensitive to neurological effects from exposure xylenes. The use of increased mortality in rats for the generation of an RfD is discounted in this assessment because the study used for this derivation notes that several of the deaths are attributed to gavage error and may not be chemically related. The study reports that there is still a dose related response, but is unclear as to the reason for the increase mortality. A supporting study that also identifies increased mortality reports increased salivation prior to and immediately following administration, and notes that the animals that died prematurely had vehicle and the administered chemical in the lungs (Wolfe, 1988b). This study attributed the increased mortality to aspiration of the administered chemical. The previous assessment also identifies hyperactivity following gavaging which is not unexpected following administration of a noxious chemical.

5.2 INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

Chronic human data are limited to the study by Uchida et al. (1993) which identifies a "concentration-related" (exposure intensity broken into 1-20 ppm and > 21 ppm) increase in

reports of eye irritation, sore throat, and a floating sensation during the work shift. Poor appetite was the only symptom to occur outside of the workplace in the previous 3-month period, however, indicating that the other effects were transient in nature and most likely due to the irritating properties of xylenes. Similar effects are reported by Klaucke et al. (1982) and Hipolito (1990). While available data indicate that xylenes are neurotoxic to humans there are no human studies that allow for derivation of an RfC. A search of the literature failed to find a chronic inhalation study. Therefore, it is considered appropriate to use subchronic animal data for the derivation of the RfC

The most consistent effect noted in the inhalation study in animals is neurotoxicity. The body of data indicates that xylenes is neurotoxic. This evidence is provided by the physical activity of the animals following administration, electrical responses, and biochemical and morphological changes. Studies by Korsak et al. (1992;1994) exhibited reduced performance on the rotarod test following exposure to xylenes. Pryor et al. (1987) and Nylen and Hagman (1994) demonstrated that inhalation exposure led to hearing impairment as indicated by a brainstem auditory evoked response test. Gralewicz et al. (1995) report effects on brain aging as indicated by spike and wave discharge activity. Biochemical and morphological effects are reported by Rosengren et al. (1986) who found that exposure to xylene led to increase in the brain concentrations of glial fibrillary acidic protein, S-100 protein and DNA: all indicators of brain injury. Using intraperitoneal administration, de Grandarias (1993, 1995) found decreases in leucine-aminopeptidases and met-enkephalin, both factors associated with neurological activity.

The principal study chosen is the study by Korsak et al. (1994). Male rats were exposed to air containing 0, 50, or 100 ppm m-xylene for 6 hours/day, 5 days/week for 3 months. Rotarod performance was decreased in a dose-response manner 50 and 100 ppm groups at one, two, and three months of exposure, with the decrease being statistically and biologically significant at 100 ppm. The LOAEL is therefore 100 ppm based on decreased rotarod performance reflecting diminished coordination, and the NOAEL is 50 ppm.

Another study by Korsak et al. (1992) also found decreased rotarod performance and decreased spontaneous activity in groups of rats exposed for 6 hours/day, 5 days/week to 100 ppm m-xylene for 6 months or 1000 ppm for 3 months as compared with controls. Other studies addressing inhalation exposures to xylene reported effects that occurred at higher exposure concentrations, or were not able to identify a LOAEL.

The choice of a study in which exposure was limited to m-xylene alone as opposed to the isomeric mixture or to one of the other individual isomers is not believed to affect the RfC derivation. As discussed in Section 4.4.3., while differences in toxicity appear among the isomers noted between studies, there are no apparent consistent, significant quantitative differences in the potency of the isomers following oral or inhalation exposure.

5.2.2. Methods of Analysis

The RfC was derived according to procedures identified in U.S. EPA (1994b) in which animal data were used to derive an equivalent exposure to humans ($NOAEL^*_{[HEC]}$). Xylene is considered a category 3 gas because of its low water solubility, potential for accumulation in blood during exposure, and nonreactivity with the tissues of the respiratory tract. Therefore, the $NOAEL^*_{[HEC]}$ was calculated using the equation:

$$NOAEL^*_{[HEC]} = NOAEL^*_{[ADJ]}(mg/m^3) \times (H_{b/g})_A/(H_{b/g})_H$$

where $NOAEL^*_{[ADJ]}$ is the NOAEL adjusted for the duration of exposure and $(H_{b/g})_A/(H_{b/g})_H$ is the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value.

The NOAEL of 50 ppm (217 mg/m^3) identified in the Korsak et al. (1994) study was therefore adjusted for duration using the following equation:

$$NOAEL^*_{[ADJ]} = (217\ mg/m^3) \times (6\ hours/24\ hours) \times (5\ days/7\ days) = 38.75\ mg/m^3.$$

The Tardif et al. (1995) study identified a $(H_{b/g})_H$ of 26.4 for m-xylene, and the Tardif et al. (1993) study identified a $(H_{b/g})_A$ of 46.0 for m-xylene in the rat; therefore:

$$(H_{b/g})_A/(H_{b/g})_H = 46.0/26.4 = 1.7$$

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

In conclusion:

$$NOAEL^*_{[HEC]} = 38.75\ mg/m^3 \times 1 = 38.75\ mg/m^3$$

Data are insufficient for analysis by the Benchmark Dose method due to the manner of reporting. Data are presented in graphical format making the reconstruction of the individual responses amenable to BMD application.

5.2.3 Inhalation Reference Concentration Derivation

An RfC of 0.13 mg/m^3 (ppm) was derived from a total uncertainty factor (UF) of 300 applied to the $NOAEL_{[HEC]}$ of 38.75 mg/m^3. The uncertainty factor includes a 3 for interspecies variation since dosimetric adjustments are made, and a 10 for intraspecies variation to account for sensitive individuals. An uncertainty factor of 3 is applied for data base insufficiency to account for limitations in studies on neurotoxicity, reproductive, and developmental effects. The RfC is derived from a subchronic study but the effects appear to be mild. As such, an uncertainty factor for subchronic to chronic data for 3 is applied. Uncertainty factors for the use of LOAEL and for database insufficiency were also not applied.

$$RfC = NOAEL_{[HEC]} \div UF$$

$$RfC = 38.75 \text{ mg/m}^3 \div 300 = 0.1 \text{ mg/m}^3$$

In previous IRIS assessment, an RfC was not derived. The generation of an RfC for this assessment is based on the availability of studies published since the last revision (U.S. EPA, 1987).

5.3 CANCER ASSESSMENT

The evidence indicates that xylenes are not likely to be carcinogenic to humans. Human epidemiological studies have found statistically increased incidence of cancer but are limited by the number of subjects in the cohort, low number of incidence reported, and confounded by exposures to other solvents. The database on animal studies of xylenes is mixed. An NTP oral carcinogenicity study found *no evidence* of carcinogenicity of xylenes in rats or mice of either sex. However, a second study by Maltoni et al. (1983, 1985) found an increase in malignant tumors following exposure to xylenes. The second study is incompletely reported and not amenable to developing a dose response. Genotoxicity studies are consistently negative.

Overall, the data indicate that xylenes is not carcinogenic to humans. Data on chemicals of similar structure such as benzene require the metabolism of the parent material to a more reactive metabolite. For benzene this involves the formation of an epoxide which is subsequently metabolized catechol and benzoquinones (U.S. EPA, 2000). This process requires the presence of two adjacent nonsubstituted carbons which is less prevalent on xylenes. Furthermore, data on the metabolism of xylenes indicate that metabolism of xylenes involves oxidation of the alkyl group to an acid that is subsequently conjugated with glycine thereby reducing the reactivity of the metabolites. Therefore, under EPA's Risk Assessment Guidelines of 1986 (U.S. EPA, 1986a), xylenes are classified into cancer weight-of-evidence Group D, *not classifiable as to human carcinogenicity*. Under the Draft Revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), xylenes are classified as *data are inadequate for an assessment of human carcinogenic potential* due to conflicting evidence.

The data which demonstrate the potential for carcinogenicity of xylenes includes epidemiological studies in which an exposed cohort demonstrates an increase in the incidence of cancer. As noted above, the epidemiological data are limited by the size of the cohort and confounded to coexposure to other solvents. As such, these data are not amendable to generating a dose response. Of the two studies that are available, the one (Maltoni et al., 1983;1985) that demonstrated carcinogenicity had several shortcomings including the use of a single dose. These data are also not amendable for generating a dose response.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1 HAZARD POTENTIAL

Xylenes is found in a number of consumer products, including solvents, paints or coatings, and as a blend in gasoline. Mixed xylenes are comprised of 3 isomers: m-xylene, o-xylene, and p-xylene, with the m-isomer predominating. Ethylbenzene is also present in the technical product formulation.

Absorbed xylene is rapidly metabolized and is excreted almost exclusively in the urine as methylhippuric acid isomers in humans and as methylhippuric acid isomers and toluic acid glucuronides in animals. Only a minor amount of xylene absorbed following inhalation exposure is excreted unchanged by the lungs, and retention by adipose tissues has been estimated to range from 3.7 to 10% of total uptake.

Effects of xylene exposure in humans include epidemiological studies of occupational exposure, case reports and acute inhalation exposures primarily addressing neurotoxicity. Central nervous system disturbances have been observed following inhalation exposure to xylene. Uchida et al. (1993) report that workers exposed to concentrations as high as 175 ppm (mean exposure 14 ppm) experienced irritation, sore throat, loss of appetite and dizziness. At higher doses there are case reports citing oral, inhalation, or intravenous xylene exposure resulting in unconsciousness/coma, acute pulmonary edema and congestion, transient renal or hepatic impairment, and death at doses reaching 10,000 ppm by inhalation (Morley et al., 1970). In a case report Abu Al Ragheb et al. (1986) report that an individual committed suicide by consuming an undisclosed amount of xylene. Death was attributed to respiratory failure and asphyxia resulting from depression in the respiratory center of the brain.

Oral exposure to xylene in animals has resulted in potential treatment-related mortality, decreases in body weight gains, renal effects (increased hyaline droplet formation in male rats; increases in the early appearance of minimal chronic nephropathy in female rats), and central nervous system disturbances. Effects of inhaled xylene in animals have included changes consistent with adaptative responses of the liver, and, most prominently, central nervous system disturbances. Exposure of pregnant animals to xylenes generally resulted in fetal toxicity only at doses similar to or exceeding those resulting in maternal toxicity.

Current data indicate that xylenes are not likely to be carcinogenic to humans. The NTP (1986) oral carcinogenicity study found *no evidence* of carcinogenicity of xylenes in rats or mice of either sex while a second study found increased incidence of cancer in laboratory animals (Maltoni et al., 1983; 1985), genotoxicity studies are consistently negative, and epidemiological studies are insufficient to support a cancer concern. Mode of action analysis based on structural similarities to benzene suggest that xylenes would be less readily metabolized to the active form (epoxide and quinones), and the metabolites that might be formed are expected to be less reactive and more readily excreted.

6.2 DOSE RESPONSE

The quantitative estimates of human risk from lifetime exposure to xylenes are based on animal experiments because no adequate human data are available. The human oral dose of xylenes that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime (RfD) is 0.7 mg/kg/day. The RfD is based upon a $NOAEL_{[ADJ]}$ derived from a subchronic toxicity study in rats (NTP, 1986), and includes a total uncertainty factor of 1000: 10 for interspecies extrapolation, 10 for intraspecies variability, and 10 for database insufficiency based on the absence of a complete neurotoxicity study and a two-generation study. Confidence in the NTP (1986) study is medium. While the NTP study (1986) investigated subchronic and chronic toxicity of xylenes in both male and female rats and mice, and includes gross necropsy and comprehensive histologic examinations, neurotoxicity, hematology, and clinical chemistry analyses were not conducted. Confidence in the overall database is medium. Chronic and subchronic toxicity studies in both male and female mice and rats and a developmental toxicity study in rats were available. Database insufficiencies include the lack of a neurotoxicity study, reproduction study and a developmental toxicity study in a second species.

The daily inhalation exposure in humans that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime (RfC) is 0.13 mg/m^3. The RfC is based upon a $NOAEL_{[HEC]}$ derived from a subchronic toxicity study in rats (Korsak et al., 1994), and includes a total uncertainty factor of 300: 3 for interspecies extrapolation and 10 each for intraspecies variability and extrapolation from subchronic to chronic exposure. Confidence in the Korsak et al. (1994) study is medium. Only males were investigated, only two tests from a multitude of standardized neurotoxicity tests were used to assess the neurotoxicity of inhaled xylene in rats, and the study is a subchronic study. Therefore, it is unknown if the pain sensitivity test and the rotarod test are the most sensitive for detecting neurological effects following xylene exposure. Despite this insufficiency, however, the LOAEL and NOAEL identified in this study are at concentrations well below the NOAEL concentrations identified in other studies. Confidence in the overall database is medium. Available studies included a chronic toxicity study in rats, a one-generation reproductive study in rats, developmental toxicity studies in rats, and subchronic toxicity studies in rats and dogs. Database deficiencies include a two-generation reproductive study, and a developmental toxicity study in a second species other than rats. An additional uncertainty factor for database insufficiencies was not applied; however, because a one-generation reproductive study was available and did not indicate toxicity, and because available data indicate that fetal toxicity occurs only at doses similar to or exceeding those resulting in maternal toxicity.

A cancer assessment is comprised of conflicting results. The human data are inadequate to generate a dose response because the exposed cohort is small, the number of reported cases is small, and the data are confounded by concurrent exposures to other solvents. Two studies involving the use of animal data produced contradictory results. The NTP (1986) study found no increased incidence of cancer in the exposed group compared with the control for male and female mice and rats. Maltoni et al. (1993, 1995) found a significant increase in the incidence of tumors in animals dosed only at 500 mg/kg/day. The application of a dose response with a single dose provides a high degree of uncertainty. This, along with additional information indicating that xylenes is not carcinogenic, leads to the decision to omit a dose response.

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