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Provisional Peer-Reviewed Toxicity Values for Xylenes (CASRN 1330-20-7)

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Commonly Used Abbreviations

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR XYLENES (CASRN 1330-20-7)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

A streamlined approach was used to derive provisional subchronic RfD and RfC values for xylenes. Xylenes have a chronic RfD, a chronic RfC, and cancer assessment on IRIS (U.S. EPA, 2008)—so only subchronic toxicity values are derived in this assessment. Xylenes have recently been reassessed by the IRIS program and a Toxicological Review (U.S. EPA, 2003) is available. In addition, the Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for xylenes has been updated recently (ATSDR, 2007). Both the IRIS Toxicological Review and the ATSDR Toxicological Profile contain comprehensive overviews of the toxicology and toxicokinetics information available on xylenes. Although all of the exposure duration-relevant studies reviewed by ATSDR (2007) are included in the 2003 IRIS review of xylenes, updated literature searches were conducted from January 2002 to July 2007 for studies relevant to the derivation of subchronic toxicity values for xylenes; Appendix C provides a description of the literature search process. No new studies with the ability to inform the derivation of subchronic provisional toxicity values were identified. As such, given the availability of the recent IRIS and ATSDR reviews, these reports were used to identify the exposure duration-relevant critical studies and endpoints for use in deriving the subchronic values.

The derivation of subchronic toxicity values for xylenes is discussed below. A brief rationale is provided for the selection of the critical study and endpoint, a summary of the critical study is presented, and the subchronic toxicity-value-derivation process is described. For further information on the toxicology and toxicokinetics of xylenes, the reader may consult the IRIS records (attached to this report as Appendix A), IRIS Toxicological Review document (U.S. EPA, 2003), or ATSDR (2007) Toxicological Profile for xylenes.

REVIEW OF PERTINENT DATA AND DERIVATION OF PROVISIONAL SUBCHRONIC TOXICITY VALUES FOR XYLENES

Subchronic p-RfD

The chronic RfD for xylenes (0.2 mg/kg-day) on IRIS (consensus date January 2003) is based on mortality and decreased body weight in a chronic rat study (NTP, 1986). The ATSDR intermediate-duration oral minimal risk level (MRL) of 0.4 mg/kg-day for mixed xylenes was derived in August 2007 based on neurological effects (hyperactivity) in a chronic mouse gavage study (NTP, 1986). NTP (1986) reported that the effects were first observed beginning in Week 4 of the study and were considered to result from intermediate-duration exposure (e.g., ATSDR considers intermediate duration to span from ≥14 days to 1 year).

All of the exposure duration-relevant studies reviewed by ATSDR (2007) are included in the 2003 IRIS review of xylenes (i.e., no new studies were published between 2003 and July 2007). Thus, the IRIS Toxicological Review was consulted to identify studies that might be relevant to the derivation of a subchronic p-RfD for xylenes (e.g., oral subchronic, developmental or reproductive toxicity studies); these studies were compared with the NTP (1986) chronic mouse study that served as the basis for the intermediate duration oral MRL for xylenes (ATSDR, 2007). Neither body weight nor survival rates (critical effects for the chronic study), used as the basis of the chronic RfD on IRIS, were affected in the first few months of the chronic rat study (NTP, 1986); as such, this study was not considered suitable for use in the derivation of a subchronic p-RfD. Table 1 compares the NOAELs, LOAELs, and endpoints of the available studies. In the table, the NOAELs and LOAELs adjusted for continuous exposure are also presented because several of the available studies (all reported by NTP, 1986) used gavage administration on 5 days/week.

Table 1. Available Studies for Subchronic p-RfD Derivation for Xylenes

Species	Sex	Doses (mg/kg-day)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Duration-Adjusted NOAEL[a] (mg/kg-day)	Duration-Adjusted LOAEL[a] (mg/kg-day)	Responses	Comments	Reference
Rat Subchronic Gavage Study	M/F	0, 62.5, 125, 250, 500, 1,000	5 days/week for 13 weeks	500	1,000	357	714	Decreased body weight in males	Mixed xylenes; included 17% ethylbenzene. Females also exhibited reduced body weight gain at the high dose	NTP, 1986
Rat Subchronic Gavage Study	M/F	0, 150, 750, 1,500	Daily for 90 consecutive days	150	750	150	750	Increased kidney weights in males and early appearance of nephropathy in females	Mixed xylenes	Condie et al., 1988
Rat Subchronic Gavage Study	M/F	0, 100, 200, 800	Daily for 90 consecutive days	200	800	200	800	Decreased body weight in males	*m*-Xylene tested. Female body weight was also reduced at the high dose	Wolfe, 1988a
Rat Subchronic Gavage Study	M/F	0, 100, 200, 800	Daily for 90 consecutive days	200	800	200	800	Early mortality in males	*p*-Xylene tested; mortality may have been related to aspiration of test material into lungs	Wolfe, 1988b
Mouse Subchronic Gavage Study	M/F	0, 125, 250, 500, 1,000, 2,000	5 days/week for 13 weeks	1,000	2,000	714	1,430	Transient signs of nervous system depression	Mixed xylenes; included 17% ethylbenzene	NTP, 1986

Table 1. Available Studies for Subchronic p-RfD Derivation for Xylenes

Species	Sex	Doses (mg/kg-day)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Duration-Adjusted NOAEL[a] (mg/kg-day)	Duration-Adjusted LOAEL[a] (mg/kg-day)	Responses	Comments	Reference
Mouse Gavage Develop-mental Toxicity	F	0, 520, 1,030, 2,060, 3,100, 4,130	Daily during GD 6–15	1,030 (maternal and develop-mental)	2,060 (maternal and develop-mental)	1,030	2,060	Decreased gravid uterine weight in dams; decreased fetal weight and increased incidence malformations in offspring	Mixed xylenes	Marks et al., 1982
Mouse Chronic Gavage Study	M/F	0, 500, 1,000	5 days/week for 103 weeks	500	1,000	357	714	Hyperactivity beginning Week 4	Mixed xylenes; included 17% ethylbenzene. This study was used as the basis for the ATSDR intermediate MRL	NTP, 1986

[a]Adjusted for continuous exposure where applicable, as follows: Adjusted NOAEL = NOAEL × 5/7 days/week. Adjusted LOAEL = LOAEL × 5/7 days/week.

As the table shows, several subchronic rat studies (NTP, 1986; Condie et al., 1988; Wolfe, 1988a,b) and the chronic mouse study (NTP, 1986) identify adjusted LOAELs in the same range (700–800 mg/kg-day). A LOAEL of 800 mg/kg-day was identified for the data in Wolfe (1988b) based on early mortality; however, U.S. EPA (2003) indicated that some of the mortality may have been related to aspiration of the test material into the lungs. Thus, this study is not considered suitable for use in deriving the subchronic p-RfD. Condie et al. (1988) identified a LOAEL of 750 mg/kg-day for increased kidney weights and early appearance of nephropathy in rats. However, as noted by U.S. EPA (2003), kidney effects were not observed in other subchronic studies in rats (NTP, 1986; Wolfe, 1988a, 1988b) nor in the chronic rat study (NTP, 1986). Consequently, this endpoint is not considered for use in deriving the subchronic p-RfD. NTP (1986) and Wolfe (1988a) identify LOAELs of 714 and 800 mg/kg-day, respectively, for decreased body weight; this endpoint is also a critical effect observed in the chronic rat study (NTP, 1986). The chronic mouse study (NTP, 1986) identifies a LOAEL of 714 mg/kg-day for transient hyperactivity beginning after 4 weeks of the study. ATSDR (2007) considered this to be an effect of intermediate duration (<1 year) exposure and used it as the basis for the intermediate oral MRL. While there are few data on the neurotoxicity of orally administered xylenes, neurological effects are a known critical hazard of inhalation exposure (for review, see Ritchie et al., 2001) and serve as the basis for the chronic RfC.

In the absence of a compelling reason to select any one of these three studies over the other (subchronic rat studies by NTP, 1986 and Wolfe, 1988a and the chronic mouse study by NTP, 1986), all three were considered as potential principal studies for the purpose of deriving a subchronic p-RfD for xylenes.

Summaries of the these studies are excerpted from the U.S. EPA (2003) Toxicological Review for Xylenes and reproduced here for the reader's convenience.

> *In the NTP (1986) subchronic rat study, groups of 10 male and 10 female Fischer 344 rats were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene and 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 62.5, 125, 250, 500 or 1000 mg/kg-day for 5 days per week for 13 weeks. At termination of the study, necropsy was performed on all animals and comprehensive histologic examinations were performed on vehicle and high dose-group animals. High-dose males and females gained 15% and 8% less body weight, respectively, than did controls, with final body weights being 89% and 97%, respectively, of those of controls (statistical significance not reported). No signs of toxicity or treatment-related gross or microscopic pathologic lesions were observed. The LOAEL is 1000 mg/kg-day, based on decreased body weights in male rats and the NOAEL is 500 mg/kg-day. After adjustment for continuous exposure, the LOAEL and NOAEL are 714 and 357 mg/kg-day, respectively.*
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> *In the study by Wolfe (1988a), groups of 20 male and 20 female Sprague-Dawley rats were administered m-xylene (99% purity) by gavage in corn oil at doses of 0, 100, 200 or 800 mg/kg-day for 90 consecutive days. Survival incidences were 20/20, 17/20, 15/20 and 18/20, respectively, for males and 20/20, 20/20, 16/20 and 16/20, respectively, for*

females. Mortality in the mid-dose males and mid- and high-dose females attained statistical significance ($p \leq 0.05$), 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 were not evident in any of the animals that survived to study termination. Histopathologic examination of the lungs from animals that died before study termination revealed foreign material in the alveoli in all but one animal. Therefore, these deaths were attributed to vehicle and/or compound aspiration. Clinical signs present throughout the study were limited to high levels of salivation prior to dosing in high-dose males and females. Body-weight gains over the entire study period were decreased ($p \leq 0.05$) in mid- and high-dose males (89% and 75%, respectively, of control weight gain) and high-dose females (85%). Food consumption was likewise decreased ($p \leq 0.05$) in high-dose males during weeks 1–5 (90% of control levels) and in mid- and high-dose males during weeks 6–9 (92% of control levels for both groups). A thorough histologic examination revealed no other abnormalities. Other effects noted were not definitively related to treatment and/or were not biologically significant. The NOAEL and LOAEL are identified as 200 and 800 mg/kg-day, respectively, based on decreased body weight in males.

In the chronic mouse study (NTP, 1986), groups of 50 male and 50 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene, 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 500 or 1000 mg/kg-day for 5 days per week for 103 weeks. Necropsy and histologic examinations were performed on all animals. Tissues were examined for gross lesions and masses. The tissues examined included mandibular lymph nodes, salivary gland, femur (including marrow), thyroid gland, parathyroids, small intestine, colon, liver, prostate/testis or ovaries/uterus, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, skin, lungs and mainstem bronchi, kidneys, adrenal glands, urinary bladder, pituitary gland, eyes (if grossly abnormal) and mammary gland. Hematology and clinical chemistry analyses were not conducted. No statistically significantly increased incidences of nonneoplastic or neoplastic lesions were found in male or female exposed groups when compared with controls. The only treatment-related effect observed was hyperactivity, which occurred in all high-dose mice of each sex 5–30 minutes after dosing. This effect was observed consistently beginning at week 4 and continued until study termination at 103 weeks. The LOAEL is 1000 mg/kg-day and the NOAEL is 500 mg/kg-day for hyperactivity. After adjustment for continuous exposure, the LOAEL and NOAEL are 714 and 357 mg/kg-day, respectively.

The potential principal studies (NTP, 1986; Wolfe, 1988a) identify LOAELs of 714 or 800 mg/kg-day and NOAELs of 200 or 357 mg/kg-day. The principal observation in the chronic mouse study (NTP, 1986) is transient hyperactivity after dosing. The incidence of this effect was 100% in both male and female mice exposed for at least 4 weeks at the LOAEL. The incidence, if any, of hyperactivity at lower doses was not reported, precluding the use of benchmark dose (BMD) modeling on this endpoint. The critical effect in the subchronic rat studies is decreased body weight (≥10% difference from controls) in male rats. The body-weight decrement was

observed in both male and female rats in two different subchronic studies (Wolfe et al., 1988a; NTP, 1986), as well as in the chronic study (NTP, 1986), and the effect exhibited dose-dependence in male rats. In addition, the chronic RfD on IRIS is based on decreased body weight in rats in the chronic NTP (1986) study. Data on decreased body weight in male rats were considered for benchmark dose modeling to derive a point of departure (POD) for the subchronic p-RfD. Modeling of the final body weights of male rats in the subchronic study reported by NTP (1986) did not result in model fit by U.S. EPA (2000) criteria (Appendix B describes the modeling approach and provides results). In contrast, modeling of the data on final body weights of male rats reported by Wolfe (1988a) using the linear model with constant variance provided adequate fit to both the variance and means data. Appendix B describes the modeling effort and results. The BMD and BMDL associated with a 10% decrease in body weight (compared with the control mean) were 538 and 440 mg/kg-day, respectively. The BMDL is selected as the POD for subchronic p-RfD derivation. A composite UF of 1000 is applied to the BMDL of 440 mg/kg-day to derive a **subchronic p-RfD** for xylenes, as shown below.

Subchronic p-RfD = BMDL ÷ UF
= 440 mg/kg-day ÷ 1,000
= **0.4 or 4×10^{-1} mg/kg/-day**

The composite UF includes a factor of 10 for interspecies extrapolation, a factor of 10 for human variability and 10 for database limitations, as follows:

- An UF of 10 is applied to account for laboratory animal-to-human interspecies differences in toxicokinetics and toxicodynamics (UF_A).
- An UF of 10 is applied for intraspecies uncertainty to account for human variability and sensitive populations (UF_H). This factor accounts for humans who may be more sensitive than the general population to exposure to xylenes.
- An UF of 1 for extrapolation from a LOAEL to NOAEL (UF_L) is applied because the current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR corresponding to a change in body-weight equal to one control standard deviation from the control mean body-weight was selected under an assumption that it represents a minimal biologically significant change.
- An UF of 10 is applied to account for database uncertainty (UF_D). The available subchronic oral database for xylenes includes subchronic gavage toxicity studies in mice and rats, and it includes a developmental toxicity study. However, the database lacks adequate studies of the oral neurotoxicity of xylenes, as well as multigenerational reproductive toxicity and developmental neurotoxicity studies. Since neurological impairment is a critical health hazard from inhalation exposure to xylenes and clinical signs of neurotoxicity have been observed after subchronic and chronic exposure in mice (NTP, 1986), the lack of comprehensive neurotoxicity testing is of particular concern.

Confidence in the principal and supporting studies is medium. The studies include two subchronic studies in rats and a chronic study (with effects observed at 4 weeks) in mice. The rat studies identified one of the same critical endpoints as was used for the chronic RfD derivation (body-weight decreases). Comprehensive histologic examinations of tissues were performed in all three studies. Confidence in the oral subchronic toxicity database is low-to-medium because the database contains several subchronic studies and an evaluation of the developmental effects, but it is lacking oral neurotoxicity studies, multigenerational reproductive toxicity studies, and developmental neurotoxicity studies. Low-to-medium confidence in the subchronic p-RfD follows.

Subchronic p-RfC

Review of the data supporting the chronic RfC for xylenes on IRIS indicate that subchronic data were used to derive the chronic value, and, thus, are appropriate to serve as the basis for the subchronic p-RfC. The chronic RfC for xylenes (0.1 mg/m^3) on IRIS (consensus date January 2003) is based on neurological effects in a subchronic rat inhalation study (Korsak et al., 1994). The derivation included a UF of 3 for subchronic-to-chronic extrapolation, which is justified because the effects did not increase with longer exposure durations. The intermediate duration inhalation MRL (0.6 ppm or 2.6 mg/m^3) was derived in August 2007 and was also based on neurological effects in the Korsak et al. (1994) study. Because U.S. EPA (2003) used a subchronic study as the basis for the chronic RfC for xylenes and ATSDR (2007), and an updated literature search did not identify any newer subchronic inhalation studies, the subchronic p-RfC is based on the same critical study (Korsak et al., 1994), endpoint (neurological effects), and POD ($NOAEL_{HEC}$) as the chronic RfC—but without the UF for subchronic-to-chronic extrapolation.

A summary of the critical study is excerpted from the U.S. EPA (2003) IRIS record for xylenes and reproduced below. Additional study details are available in the U.S. EPA (2003) Toxicological Review for xylenes.

> *Korsak et al. (1994) exposed groups of 12 male Wistar rats by inhalation to 0, 50 or 100 ppm m-xylene or n-butyl alcohol or a 1:1 mixture (purity of chemicals not provided) for 6 hours per day, 5 days per week for 3 months and evaluated similar endpoints as in the earlier study (Korsak et al., 1992). Rotarod performance and spontaneous motor activity were assayed. The report does not specify the timing of the neurologic examinations; however, given that the 1994 study was conducted by the same group of investigators as a 1992 study (Korsak et al., 1992) and that one of the tests (rotarod performance) was the same in both studies, it appears reasonable to assume that the tests were administered 24 hours after termination of exposure. The rotarod test was used as a measure of motor coordination disturbances from exposure to m-xylene. The rotarod test involves placing the subject animals on a rotating rod and evaluating their ability to remain on the rod for a period of 2 minutes. The animals were trained to perform the task, exposed to chemical or control gas and evaluated at defined intervals. By the time interval after exposure, considerable proportions of absorbed xylenes are expected to have been eliminated from the body (see Toxicological Review, U.S. EPA, 2003). Body weights and weights of seven organs were measured. Blood for clinical biochemistry*

(e.g., alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, alkaline phosphatase and total protein) and hematologic analysis (erythrocyte counts, hemoglobin concentration, hematocrit, leukocyte count and differential leukocyte counts) was collected 24 hours after termination of exposure. Statistical evaluations (using a p = 0.05 level of significance) of the collected data included analysis of variance, Dunnett's test and Fisher's exact test.

No statistically significant exposure-related changes were noted in body-weight gain, absolute or relative organ weights, hepatic activities of microsomal monooxygenases, lipid peroxidation or levels of triglycerides in the liver (Korsak et al., 1994). Statistically significant decreases in erythrocyte number were seen in animals exposed to 50 ppm (93% of controls) or 100 ppm (80.5% of controls) of m-xylene alone. Similarly, decreased levels of hemoglobin were reported in both groups (92% of controls for both groups). At 100 ppm, a statistically significant increase in leukocyte number (35% increase over controls) was reported. Exposure to 50 or 100 ppm m-xylene alone also resulted in decreased rotarod performance starting at 1 month of exposure, which remained at the same level until the end of the 3-month exposure. Decreases were statistically significant in the 100 ppm group when compared with the controls. The results were presented in graphical form; the actual numerical data are not provided. The decreases in performance were roughly 8% and 33% for the 50 and 100 ppm groups, respectively, versus 0% for the controls.

Sensitivity to pain was assessed using the hot plate behavior test, in which the animals are placed on a hot (54ºC) surface and the time interval between being placed on the plate and licking of the paws is measured (Korsak et al., 1994). Rats exposed to 50 or 100 ppm m-xylene alone had statistically significantly increased sensitivity to pain at the end of the 3-month exposure (latency of the paw-lick response was 8.7 and 8.6 seconds, respectively, vs. 12.2 seconds for the controls). The LOAEL is 100 ppm, based on decreased rotarod performance and decreased latency in the paw-lick response in the hot-plate test and the NOAEL is 50 ppm.

As noted above, the subchronic p-RfC is based on the same critical study, effect, and POD as the IRIS chronic RfC. For the chronic RfC, U.S. EPA (2003) used the $NOAEL_{HEC}$ (39 mg/m^3) calculated from the data reported by Korsak et al. (1994) as the POD. The $NOAEL_{HEC}$ was divided by a composite UF of 300 that includes a 3-fold UF for interspecies extrapolation (dosimetric adjustments were used to extrapolate the toxicokinetic portion), a 10-fold UF for intraspecies variation, a 3-fold UF for extrapolation from subchronic-to-chronic exposure duration and a 3-fold UF for database deficiencies (reflecting a lack of multigeneration reproductive toxicity study). Further detail on the UF selections is available in the IRIS record (see Appendix A).

For the derivation of a subchronic p-RfC, the $NOAEL_{HEC}$ of 39 mg/m^3 was divided by a composite UF of 100, resulting in a **subchronic p-RfC calculated as follows:**

Subchronic p-RfC = $NOAEL_{HEC} \div UF$
= $39\ mg/m^3 \div 100$
= **0.4 or 4×10^{-1} mg/m^3**

The uncertainty factors included in the composite UF are the same as those used for the chronic RfC—but without the UF for subchronic-to-chronic extrapolation. The composite UF of 100 applied here includes a 3-fold UF for interspecies extrapolation (toxic dynamic portion only), a 10-fold UF for intraspecies variation, and a 3-fold UF for database deficiencies as follows:

- An UF of 3 is applied to account for laboratory animal-to-human interspecies differences (UF_A). A factor of 3 is applied because default $NOAEL_{HEC}$ dosimetric adjustments were used to calculate a human equivalent concentration (HEC), reducing the uncertainty involved with the extrapolation from the results of an animal study to a human exposure scenario (i.e., the toxicokinetic portion of the UF is 1; the toxicodynamic portion of the UF is 3).
- An UF of 10 is applied for intraspecies uncertainty to account for human variability and sensitive populations (UF_H). The degree of human variance in abilities to absorb or dispose of xylenes is unknown, as is the degree of human variance in responding to xylenes neurotoxicity. Results from developmental toxicity studies of rats exposed by inhalation during gestation indicate that untoward developmental effects occur only at higher doses than chronic doses producing the critical effects observed in adult male rats in the principal and supporting studies. This suggest that the developing fetus is not at special risk from low-level exposure to xylenes (please refer to the IRIS Toxicological Review of Xylenes for details). However, as with oral exposure, the effects of inhaled xylenes in other potentially sensitive populations such as newborns or young children or animals have not been assessed.
- An UF of 3 is applied for uncertainties in the database (UF_D). The inhalation database includes some human studies, subchronic studies in rats and dogs, neurotoxicity studies, a one-generation reproductive toxicity study, developmental toxicity studies, and developmental neurotoxicity studies. Although the available developmental toxicity studies are confounded by a lack of litter incidence reporting, the data reported for fetal incidences do not indicate effects at levels lower than that found to induce neurologic impairment in several endpoints in male rats (please refer to the IRIS Toxicological Review of Xylenes for details). The database is lacking a two-generation reproductive toxicity study.

As discussed further in the IRIS Summary and Toxicological Review for xylenes, confidence in the principal study is medium because the study examined a broad array of endpoints including the critical effect of xylenes. However, only one sex of a single species was examined and histologic examination of the animals was not performed. Confidence in the database is medium, as it contains several subchronic studies as well as several developmental

studies, developmental neurotoxocity studies, and a one-generation reproductive toxicity study; however, a two-generation reproduction study is not available. As such, confidence in the subchronic p-RfC is medium.

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APPENDIX A: PERTINENT SECTIONS FROM IRIS SUMMARY FOR XYLENES: CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

Xylenes; CASRN 1330-20-7

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Xylenes

File First On-Line 09/30/1987

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	02/21/2003
Inhalation RfC Assessment (I.B.)	on-line	02/21/2003
Carcinogenicity Assessment (II.)	on-line	02/21/2003

_I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

_I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Xylenes
CASRN — 1330-20-7
Last Revised — 02/21/2003

The oral Reference Dose (RfD) is based on the assumption that thresholds generally exist for certain toxic effects such as cellular necrosis. 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 effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

The RfD in this updated assessment replaces a previous RfD value of 2 mg/kg-day. The previous and new RfD values are based on the same principal study (NTP, 1986). A database uncertainty factor (UF) was not considered in the derivation of the previous RfD.

The term xylenes refers to mixtures of the three xylene isomers (o-, m-, p-) and ethylbenzene. m-Xylene is commonly the predominant component (40-77%) in commercial preparations of xylenes (also referred to as mixed xylenes), with the other components each comprising roughly up to 20% of the mass. The use of xylenes as a solvent, in paints and coatings, and in gasoline is widespread. For the most part, studies cited in this assessment are conducted on mixed xylenes. Results from studies comparing the toxicity of individual xylene isomers indicate that differences, when they occur, are specific to the endpoint under consideration (see Section 4.4.3 of the Toxicological Review for more information).

__I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased body weight, increased mortality Chronic F344/N rat study Oral gavage exposure (NTP, 1986)	NOAEL: 250 mg/kg-day (179 mg/kg-day)* LOAEL: 500 mg/kg-day	1000	1	0.2 mg/kg-day

*Conversion Factors and Assumptions — 250 mg/kg-day x 5 days/7 days = 179 mg/kg-day.

__I.A.2. Principal and Supporting Studies (Oral RfD)

The National Toxicology Program's 2-year study in rats was selected as the principal study and the subchronic toxicity studies in rats by Wolfe (1988a, b) as supporting studies. In the NTP (1986) study, groups of 50 male and 50 female Fischer 344 rats and 50 male and 50 female B6C3F1 mice were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 9.1% o-xylene, 17.0% ethylbenzene) in corn oil by gavage at doses of 0, 250, or 500 mg/kg-day (rats) and 0, 500, or 1000 mg/kg-day (mice) for 5 days per week for 103 weeks. Necropsy and histologic examinations were performed on all animals. Tissues were examined for gross lesions and masses. The tissues examined included mandibular lymph nodes, salivary gland, femur (including marrow), thyroid gland, parathyroids, small intestine, colon, liver, prostate/testis or ovaries/uterus, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, skin, lungs and mainstem bronchi, kidneys, adrenal glands, urinary bladder, pituitary gland, eyes (if grossly abnormal), and mammary gland. Hematology and clinical chemistry analyses were not conducted.

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

11/50, respectively, for the control, low-, and high-dose groups). The authors did not record observations of rat behavior during dosing. Based on the available observations, the incidence of treatment-related deaths demonstrated a dose-related increase (11/50, 17/50, and 19/50, respectively [22%, 34%, and 38%]). The LOAEL is 500 mg/kg-day and the NOAEL is 250 mg/kg-day for decreased body weight and decreased survival. There was no evidence of carcinogenicity in male or female rats exposed to doses up to 500 mg/kg-day.

In mice, the only treatment-related effect observed was hyperactivity, which occurred in all high-dose mice of each sex, 5-30 minutes after dosing. This effect was observed consistently beginning at week 4, and it continued until study termination at 103 weeks. The LOAEL is 1000 mg/kg-day and the NOAEL is 500 mg/kg-day for hyperactivity.

In a study by Wolfe (1988a), groups of 20 male and 20 female Sprague-Dawley rats were administered m-xylene (99% purity) by gavage in corn oil at doses of 0, 100, 200, or 800 mg/kg-day for 90 consecutive days. Survival incidences were 20/20, 17/20, 15/20, and 18/20, respectively, for males, and 20/20, 20/20, 16/20, and 16/20 for females. Mortality in the mid-dose males and mid- and high-dose females attained statistical significance ($p <= 0.05$), 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 ($p <= 0.05$) in mid- and high-dose males (89% and 75% of controls', respectively) and high-dose females (85% of controls'). Food consumption was likewise decreased ($p <= 0.05$) in high-dose males during weeks 1-5 (90% of control levels) and in mid- and high-dose males during weeks 6-9 (92% of control levels for both groups). A thorough histologic examination revealed no other abnormal findings. Other effects noted were not definitively related to treatment and/or were not biologically significant. The NOAEL and LOAEL are identified as 200 and 800 mg/kg-day, respectively, based on decreased body weight.

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

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

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

__I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 1000

A UF of 10 was applied to account for laboratory animal-to-human interspecies differences. No information is available to support a change from default.

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

A UF of 10 was used to account for database uncertainty. The available oral database for xylenes includes chronic and subchronic gavage toxicity studies in mice and rats and a developmental toxicity study. None of these studies indicate that additional data would result in a lower RfD. However, the database lacks adequate studies of the oral neurotoxicity of xylenes as well as multigenerational reproductive toxicity and developmental neurotoxicity studies. Given the identification of neurological impairment as a critical health hazard from inhalation exposure to xylenes, the lack of comprehensive neurotoxicity testing following chronic oral exposure is of particular concern. It should be noted that transient neurotoxic effects (e.g., lethargy, tremors and unsteadiness) were reported in mice following oral exposure to xylenes for 13 weeks (NTP, 1986). There are no toxicokinetic data identifying oral dose levels at which first-pass hepatic metabolism of xylenes becomes saturated in animals or humans; such data could decrease uncertainty regarding whether neurological impairment may occur at dose levels below those causing body weight decreases and mortality in rats. It is uncertain whether the availability of comprehensive oral neurotoxicity data would result in a lower RfD.

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

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

MF = 1

__I.A.4. Additional Studies/Comments (Oral RfD)

In a NTP (1986) study, groups of 10 male and 10 female Fischer 344 rats were administered mixed xylenes (60% m-xylene, 13.6% p-xylene, 17.0% ethylbenzene, 9.1% o-xylene) in corn oil by gavage at doses of 0, 62.5, 125, 250, 500, or 1000 mg/kg-day for 5 days per week for 13 weeks. At termination of the study, necropsy was performed on all animals and comprehensive histologic examinations were performed on vehicle and high-dose group animals. High-dose males and females gained 15% and 8% less body weight, respectively, than did controls, with final body weights being 89% and 97%, respectively, of those of controls (statistical significance not reported). No signs of toxicity or treatment-related gross or microscopic pathologic lesions were observed. The LOAEL is 1000 mg/kg-day and the NOAEL is 500 mg/kg-day based on decreased body weight in male rats without tissue lesions.

In the same study, male and female $B6C3F_1$ mice were treated with mixed xylenes. Groups of 10 mice of each sex were administered 0, 125, 250, 500, 1000, and 2000 mg/kg-day in corn oil by gavage for 5 days per week for 13 weeks. Two female mice in the high-dose group died prematurely, although gavage error could not be ruled out as the cause. At 2000 mg/kg-day, starting 5-10 minutes after dosing and lasting for 15-60 minutes, the animals exhibited lethargy, short and shallow breathing, unsteadiness, tremors, and paresis. In the high-dose group, mean body weight was 7% lower for males and 17% lower for females than in the vehicle control. Although not stated explicitly, the text implies that this was a common finding among the animals dosed at this level. No treatment-related gross or microscopic pathologic lesions were seen in this study. The NOAEL is 1000 mg/kg-day and the LOAEL is 2000 mg/kg-day for transient signs of nervous system depression in mice without tissue lesions.

In a study by Condie et al. (1988) groups of 10 male and 10 female Sprague-Dawley rats were administered mixed xylenes (17.6% o-xylene, 62.3% m-xylene and p-xylene [which coeluted], 20% ethyl benzene) by gavage in corn oil for 90 consecutive days at doses of 0, 150, 750, or 1500 mg/kg-day. Effects of exposure included decreased body weights in high-dose males (94% of controls'), dose-related increased liver weights and liver-to-body weight ratios in all exposed groups of males (8, 18, and 29% increase in absolute weight above controls' in the low-,

mid-, and high-dose animals, respectively) and in mid- and high-dose females (14 and 30%, respectively), and increased kidney weights and kidney-to-body weight ratios in mid- and high-dose males (16 and 19% increase in absolute weight relative to controls', respectively) and high-dose females (18% increase in absolute weight relative to controls). The authors postulated that the modest increases in aspartate aminotransferase seen in high-dose females and increases in alanine aminotransferase in high-dose males and in mid- and high-dose females, combined with the lack of significant histopathologic findings in the liver, suggest that the enlargement of the liver was an adaptation response to xylenes 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 in female rats (1/10, 3/10, 6/10, 7/10, respectively). Compared with controls, the incidence of minimal nephropathy was statistically significantly elevated (p<0.05) in the 750 and 1500 mg/kg-day female groups but not in the 150 mg/kg-day group (Fishers exact test performed by Syracuse Research Corporation). The hyaline droplet formation in male rats was assumed by the authors to be related to male rat-specific a-2µ-globulin accumulation and not to be relevant to humans. The LOAEL is 750 mg/kg-day, based on increased kidney weights and early appearance of mild nephropathy in female rats, and the NOAEL is 150 mg/kg-day.

Kidney effects were not found in the NTP (1986) bioassay with Fisher 344/N rats or B6C3F1 mice exposed to xylenes for 13 weeks or 2 years. Likewise, no nephropathy was reported in a nephrotoxicity screening assay in male Fischer 344/N rats exposed to 2000 mg/kg m-xylene for 5 days per week for 4 weeks (Borriston Laboratories, Inc., 1983). In addition, no kidney effects were found in Sprague-Dawley rats exposed for 90 days to m-xylene or p-xylene at doses as high as 800 mg/kg-day (Wolfe et al., 1988a, b). Thus, the available data do not consistently identify the kidney as a sensitive target of xylenes in animals. Likewise, the available data do not consistently identify the liver as a sensitive target of xylenes in animals (NTP, 1986; Wolfe et al., 1988a, b; Condie et al., 1988).

A developmental toxicity study in CD-1 mice (Nawrot and Staples, 1980) indicates that developmental effects may occur following exposure to xylenes. However, the study was reported as an abstract with incomplete documentation of exposure protocols and results; it does not identify reliable NOAELs and LOAELs for maternal and developmental toxicity. Nevertheless, information in the abstract indicates that exposure on gestation days 6-15 to daily doses of o-, m-, or p-xylene at 1935 or 2580 mg/kg-day-but not at 774 mg/kg-day-resulted in overt maternal toxicity and increased incidences of cleft palate in the fetuses.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__I.A.5. Confidence in the Oral RfD

Study — Medium
Database — Medium
RfD — Medium

Confidence in the principal study is medium. The study was a 2-year toxicology and carcinogenesis assay that evaluated the critical endpoint for RfD derivation (body weight and mortality) and included comprehensive histologic examination of tissues for nonneoplastic and neoplastic lesions. Some gavage errors occurred during the study, limiting the confidence assessment to medium. Confidence in the oral exposure database is medium because the database contains chronic animal studies in two species (rats and mice), numerous subchronic studies, and an evaluation of the developmental effects of oral xylenes, but it is lacking oral neurotoxicity studies as well as multigenerational reproductive toxicity and developmental neurotoxicity studies. Medium confidence in the RfD follows.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.A.6. EPA Documentation and Review of the Oral RfD

Source Document - U.S. EPA, 2002a

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA (2002a). ***To review this appendix, exit to the toxicological review, Appendix A, Summary of and Response to External Peer Review Comments (PDF)***

Agency Consensus Date - 01/30/2003

__I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

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_I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Xylenes
CASRN — 1330-20-7
Last Revised — 02/21/2003

The inhalation Reference Concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/cu.m. In general, the RfC is an estimate (with uncertainty spanning perhaps an order

of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

As noted in Section I.A. of this file, xylenes refers to mixtures of all three xylene isomers and ethylbenzene. The inhalation RfC for xylenes presented herein is based on a principal study (Korsak et al., 1994) in which rats were exposed by inhalation to m-xylene. There is some uncertainty associated with selecting a principal study for xylenes that involved exposure to m-xylene alone, but this isomer is generally predominant in commercial mixtures. In addition, although there are no studies comparing xylene isomers in affecting critical neurological endpoints following subchronic or chronic inhalation exposure, the potencies of individual xylene isomers were similar in affecting neurobehavior, as shown in a study of rats following acute exposures (Moser et al., 1985) (see Section 4.4.3 of the Toxicological Review for more information).

No inhalation RfC for xylenes has previously been on IRIS.

__I.B.1. Inhalation RfC Summary

Critical Effect	Experimental Doses*	UF	MF	RfC
Impaired motor coordination (decreased rotarod performance) Subchronic inhalation study in male rats (Korsak et al., 1994)	NOAEL: 50 ppm $NOAEL_{(HEC)}$: 39 mg/m^3 LOAEL: 100 ppm $LOAEL_{(HEC)}$: 78 mg/m^3	300	1	0.1 mg/m^3

*Conversion Factors and Assumptions - MW = 106.17. Assuming 25C and 760 mmHg, NOAEL(mg/m^3) = 50 ppm x 106.17/24.45 = 217 mg/m^3. $NOAEL_{[ADJ]}$ = 217 mg/m^3 x 6 hrs/day x 5 days/7 days = 39 mg/m^3. The $NOAEL^*_{HEC}$ was calculated for extrarespiratory effects of a Category 3 gas (U.S. EPA, 1994). Blood/gas partition coefficients: $H(b/g)_{rat}$ = 46.0; $H(b/g)_{human}$=26.4 (Tardif et al., 1995). $(H_{b/g})_{rat}/(H_{b/g})_{human}$ =1.7; value of 1 is used when the ratio is >1 (U.S. EPA, 1994). $NOAEL^*_{HEC}$ = $NOAEL_{[ADJ]}$ x $(H_{b/g})_{rat}/(H_{b/g})_{human}$ = 39 mg/m^3.

__I.B.2. Principal and Supporting Studies (Inhalation RfC)

Korsak et al. (1992) exposed groups of 12 male Wistar rats to toluene, m-xylene, or a 1:1 mixture for 6 hours per day, 5 days per week at a concentration of 0 or100 ppm for 6 months or 1000 ppm for 3 months. Rotarod performance and spontaneous motor activity were assayed 24 hours after termination of the exposure periods. The rotarod test was used as a measure of

motor coordination disturbances from exposure to m-xylene. The rotarod test involves placing the subject animals on a rotating rod and evaluating their ability to remain on the rod for a period of 2 minutes. The animals were trained to perform the task, exposed to chemical or control gas, and evaluated at defined intervals. By the time interval after exposure, considerable proportions of absorbed xylenes are expected to have been eliminated from the body (see Section 3.4 and Appendix B of the Toxicological Review).

Body weights and weights of seven organs were measured; only data for animals sacrificed after 3 months of exposure was reported (controls and 1000 ppm rats). At 3 and 6 months, blood samples were collected 24 hours after termination of exposure for measurement of serum chemistry variables (e.g., alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, and total protein) and hematologic variables (erythrocyte counts, hemoglobin concentration, hematocrit, leukocyte count, and differential leukocyte counts). Serum chemistry and hematologic results were reported only for rats exposed to 1000 ppm for 3 months. Statistical evaluations (using a p=0.05 level of significance) of collected data included analysis of variance, Dunnet's test, and Fishers exact test.

Rats exposed to m-xylene alone exhibited statistically significantly decreased rotarod performance and decreased spontaneous activity, as measured 24 hours after termination of the exposures, when compared with controls. The percentages of failures in the rotarod test were roughly 60% in rats exposed to 1000 ppm for 3 months, 35% in rats exposed to 100 ppm for 6 months, and 0% for controls at either time period. The mean spontaneous motor activity in rats exposed to 100 ppm for 6 months was about 400 movements per hour, compared with about 800 movements per hour for controls. Spontaneous motor activity data for rats exposed to 1000 ppm m-xylene for 3 months were not presented in the report. No statistically significant exposure-related changes in body weight, absolute or relative organ weights, or clinical chemistry or hematology variables were noted in rats exposed to 1000 ppm m-xylene for 3 months, with the exception of decreased differential counts (percentage of white blood cells counted) of lymphocytes (45.5 ± 9.5 vs. 60.8 ± 6 .4 for controls; 25% decrease) and increased counts of monocytes (16.3 ± 8.9 vs. 8.3 ± 4.2 for controls; 96% increase). Total counts of white blood cells (in units of cells per mm^3 of blood), however, were not statistically significantly changed by exposure. The LOAEL is 100 ppm, based on decreased rotarod performance and decreased spontaneous motor activity. No NOAEL was identified.

In a second study, Korsak et al. (1994) exposed groups of 12 male Wistar rats by inhalation to 0, 50, or 100 ppm m-xylene or n-butyl alcohol or a 1:1 mixture (purity of chemicals not provided) for 6 hours per day, 5 days per week, for 3 months and evaluated similar endpoints as in the earlier study (Korsak et al., 1992). Blood for clinical biochemistry and hematologic analysis was collected 24 hours after termination of exposure. The report does not specify the timing of the neurologic examinations; however, given that the 1994 study was conducted by the same group of investigators as the 1992 study and that one of the tests (rotarod performance) was the same in both studies, it appears reasonable to assume that the tests were administered 24 hours after termination of exposure. Statistical evaluations (using a p=0.05 level of significance) of the collected data included analysis of variance, Dunnet's test, and Fishers exact test.

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

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

To evaluate whether xylenes influence aging of the central nervous system or induces persistent changes in radial maze performance, Gralewicz et al. (1995) exposed 8-month-old, male LOD-Wistar rats (20 per dose level) to air containing 0, 100, or 1000 ppm "pure" m-xylene (exact purity not provided) for 6 hours per day, 5 days per week, for 3 months. One-hour electroencephalograph (EEG) recordings were performed on days 28 and 56 of exposure and on days 14, 28, 56, and 84 after exposure. The number and duration of spontaneous neocortical spike and wave discharges (SWD) from the EEG were taken as electrophysiological indices of the biological age of the brain. As rats age, SWDs increase in number and become longer. Because of large interindividual variation in number and duration of SWDs within each group, these variables were normalized to a percentage of the initial values. Exposed rats were not subjected to the daily exposure protocol when EEG recordings were made on days 28 and 56 during the exposure period. Tests of spatial learning in an 8-arm radial maze were also conducted for a 2-week period starting from day 70 after exposure to day 83.

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

The analysis of variance indicated no group effect on the normalized number and cumulative-duration SWD variables. However, a statistically significant group x successive recording period effect was indicated. In control rats, these variables were increased to a

statistically significant degree, compared with those of the exposed groups, only on day 84 after exposure. The mean cumulative SWD duration (expressed in percentage) on day 84 was about 300 for the control compared with means of about 150 in each of the exposed groups. The authors hypothesized that these exposure-related changes in the spontaneous, age-related changes in cortical SWD activity may be related to cortical excitability or to an increase in catecholaminergic transmissions.

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

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

In the open-field activity test, animals were placed in a 100 cm x 100 cm arena that was surrounded by 20 cm high walls and divided into 49 equal squares. The number of square borders crossed (locomotor activity), number of rearings (exploratory activity), and number of grooming episodes were recorded. In the passive avoidance test, animals were placed on a platform above the floor of the cage, and the time until the animal stepped off the platform was recorded in a series of six trials. In the first two trials, the animals were allowed to explore the cage for 60 seconds after stepping down; in the third trial, the animals received a series of footshocks after stepping off the platform. In trials 4, 5, and 6 the animals received no shocks and were allowed to stay on the floor for 1 minute after stepping off the platform. In the active avoidance test, animals were trained to avoid an electric footshock by moving from one compartment of the cage to another when a sound is played. After successfully displaying avoidance behavior in four of five trials, the animals were considered to be trained. Post-exposure evaluations determined the frequency of avoidance behavior in response to the same stimulus.

No differences between control and exposed rats were seen in radial maze parameters (number of arm entries, arms omitted, or arms entered multiple times) either before exposure (7 days prior to

exposure) or at 14-18 days after the termination of exposure. Similarly, no differences in open-field activity were seen between groups examined on day 8 prior to exposure or day 25 postexposure or in active avoidance (number of trials to avoidance criterion), examined on days 54 and 60 post-exposure. Xylene-exposed rats showed a significantly shorter step-down time (trial 6 only; no difference in trials 1-5) in the passive avoidance test (examined on days 39-48 postexposure) and a significantly greater paw-lick latency in the hot plate behavior test (examined on days 50-51 postexposure), identifying 100 ppm as a LOAEL for neurobehavioral effects.

Because available human data are insufficient for deriving an RfC and chronic animal inhalation data are lacking, the subchronic study of Korsak et al. (1994) was selected as the principal study and Korsak et al. (1992), Gralewicz et al. (1995), and Gralewicz and Wiaderna (2001) as the supporting studies. Neurological effects (impaired motor coordination) are selected as the critical effect for deriving the RfC. Two neurological endpoints were evaluated in this study. Rotarod performance was statistically significantly decreased (33% from controls') at 100 ppm, and a statistically significant decreased sensitivity to pain was observed at 50 and 100 ppm (8.6 and 8.7 seconds, respectively, vs. 12.2 seconds for controls; measurements made 24 hours postexposure). Gralewicz and Wiaderna (2001) also measured the effect of m-xylene exposure (6 hrs/day, 5 days/wk for 4 weeks; neurological endpoints measured postexposure day 50) on pain sensitivity. In this study, a statistically significant increase in pain sensitivity (35 seconds vs. 10 seconds in control) was found at the 100 ppm dose, the lowest dose tested. The variation in the response to m-xylene in these two studies decreases the confidence in using the pain sensitivity endpoint as the critical effect.

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

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

__I.B.3. Uncertainty and Modifying Factors (Inhalation RfC)

UF = 300

A UF of 3 was applied to account for laboratory animal-to-human interspecies differences. A factor of 3 was applied because default $NOAEL_{HEC}$ dosimetric adjustments were used to calculate a human equivalent concentration (HEC), reducing the uncertainty involved with the extrapolation from the results of an animal study to a human exposure scenario (i.e., the toxicokinetic portion of the UF is 1; the toxicodynamic portion of the UF is 3).

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

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

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

MF = 1

__I.B.4. Additional Studies/Comments (Inhalation RfC)

The weight of evidence from limited human data and more extensive animal data identify mild neurological impairment and possible developmental effects as potential health hazards from repeated inhalation exposure to xylenes. The animal inhalation exposure database contains no chronic toxicity studies, but there are a number of subchronic toxicity studies (of which several focused on neurological endpoints), a one-generation reproduction study in rats, and several developmental toxicity studies, some of which evaluated offspring for performance in neurobehavioral tests. Subchronic toxicity assays in animals have not found consistent evidence for other noncancer effects, such as changes in body weight or in hepatic, hematologic, or renal toxicity endpoints, following exposure to concentrations of xylenes as high as 800-1000 ppm for

6 hours per day, 5 days per week (e.g., Carpenter et al., 1975; Jenkins et al., 1970; Korsak et al., 1992, 1994).

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

In other studies involving single or multiple 4-hour exposures of human volunteers to 200 ppm xylenes, reversible effects on balance and reaction times have been reported (Laine et al., 1993; Savolainen and Linnavuo, 1979; Savolainen et al., 1984); however, other studies of 4-hour exposures to 200 ppm have not found impaired performance in tests of simple reaction time, short-term memory, and choice reaction time (Olson et al., 1985) or changes in visually evoked brain potentials (Seppäläinen et al., 1983) or electroencephalographic patterns (Seppäläinen et al., 1991). Impaired performance on tests of memory and reaction times was also reported for subjects exposed to 100 ppm xylenes for 4 hours (Dudek et al., 1990). The available controlled-exposure human studies indicate that concentrations around 100-200 ppm are close to the threshold level for short-term reversible neurological and irritation effects from xylenes.

The available human data alone do not provide adequate evidence for neurological impairment from repeated exposure to xylenes concentrations less than or equal to 200 ppm. Aside from the controlled-exposure studies reviewed above, most of the human data associating xylenes exposure to neurological impairment are case reports involving acute high-level exposures (800-10,000 ppm) (e.g., Goldie, 1960; Hipolito, 1980; Klaucke et al., 1982). Epidemiologic studies are restricted to a cross-sectional health evaluation study (Uchida et al., 1993) that reported increased prevalence of self-reported neurological symptoms and irritation, but no apparent changes in serum enzymes indicative of liver or kidney damage in a group of Chinese workers. The workers were from a boot manufacturing plant that used a xylene-containing glue and two other plants that used mixed xylenes as a solvent in wire production or printing. The measured time-weighted-average mean concentration of airborne xylenes in these workplaces was 21 (± 21) ppm. The study has several limitations, including a lack of reporting on the duration of exposure, co-exposure to other chemicals, no clear demonstration of relationships between response and dose or duration, and the inherent bias presented by self-reporting of symptoms.

Although the human evidence for persistent effects on the nervous system or other persistent effects from repeated inhalation exposure to xylenes is inadequate, results from animal studies more clearly identify potential persistent neurological impairment and possible developmental effects as potential health hazards from repeated inhalation exposure.

Overall results from rat studies described in Section I.B.2 provide evidence that repeated exposure to m-xylene at concentrations >= 100 ppm (6 hrs/day, 5 days/wk) may produce persistent changes in several neurologic endpoints in adult rats. Supporting evidence for potential persistent neurologic effects from xylenes includes reports of changes in indices of hearing loss in rats exposed to >= 800 ppm mixed xylenes for 14 hours per day for 6 weeks (Pryor et al., 1987) and in rats exposed to 1000 ppm mixed xylenes for 18 hours per day, 7 days per week, for 61 days (Nylén and Hagman, 1994).

There are no studies of the possible developmental toxicity of inhaled xylenes in humans, but there are a number of studies examining standard developmental toxicity endpoints and neurobehavioral endpoints in offspring of animals exposed to mixed xylenes or individual xylene isomers. Evidence for impaired neurological development in rat offspring following gestational exposure to inhaled xylenes is not strong or consistent. Changes in neurobehavioral variables reported for offspring of animals exposed during gestation are restricted to impaired cognitive but not motor performance in the Morris water maze test in female but not male offspring of rats exposed to 500 ppm mixed xylenes for 6 hours per day on gestation days 7-20 (Hass et al., 1995, 1997) and decreased rotarod performance in offspring of rats exposed to 200 ppm "technical" xylenes for 6 hours per day on gestation days 6-20 (Hass and Jakobsen, 1993). Deficits in the water maze test were only observed in female rat offspring raised in standard housing and not in female rats raised in "enriched" housing with various toys (Hass et al., 1995).

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

Several other inhalation developmental toxicity studies have examined standard developmental toxicity endpoints in rats (Litton Bionetics, 1978; Bio/dynamics Inc., 1983; Rosen et al., 1986; Ungváry et al., 1980; Ungváry and Tátrai, 1985), mice (Ungváry and Tátrai, 1985) and rabbits (Ungváry and Tátrai, 1985) following gestational exposure to xylenes. These studies have most clearly identified maternally toxic levels for decreased body weight gain in pregnant rats at concentrations greater than or equal to 700 ppm o-, p-, or m-xylene for 24 hours per day (Ungváry et al., 1980) or 1600 ppm p-xylene for 6 hours per day (Rosen et al., 1986) and for maternal death and abortions in pregnant rabbits exposed to 230 ppm (but not 115 ppm) mixed xylenes or p-xylene for 24 hours per day (Ungváry and Tátrai, 1985). In rats, effects on fetal skeletal and visceral malformations (such as cleft palate) and variations (such as retarded skeletal ossification or extra ribs) were reported at concentrations of up to 700 ppm o-, m-, or p-xylene for 24 hours per day (Ungváry et al., 1980) or 780 ppm mixed xylenes for 24 hours per day (Bio/dynamics Inc., 1983; Litton Bionetics, 1978; Ungváry and Tátrai, 1985). Likewise, effects on skeletal and visceral malformations and variations were reported in mice at concentrations of up to 230 ppm mixed xylenes (12 hrs/day in three 4-hr periods) or 115 ppm o-, p-, or m-xylene

by the same protocol (Ungváry and Tátrai, 1985) or in rabbits exposed to 115 ppm mixed xylenes or o-, p-, or m-xylene for 24 hours per day (Ungváry and Tátrai, 1985).

Statistically significant increased incidences of fetuses with retarded skeletal ossification or extra ribs were reported in these studies, but the incidences were reported on an exposure-group basis in all but one of the studies. No litter-specific information was provided except in the Litton Bionetics (1978) study, which reported that, after adjustment for covariance with litter size, incidences of fetuses with delayed ossification in rats exposed to 400 ppm were no longer statistically significantly different from control values.

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

Information regarding the potential reproductive toxicity of xylenes in humans is restricted to case-control studies reporting possible associations between occupational exposure to xylenes and other solvents and spontaneous abortions (e.g., Taskinen et al., 1986, 1994). However, these studies are of limited usefulness in assessing the potential reproductive toxicity of xylenes, because the numbers of cases of spontaneous abortions were small, and the women had been exposed to a number of chemicals.

Two reproductive toxicity studies in rats exposed to xylenes by inhalation are available (Bio/dynamics Inc., 1983; Nylén and Hagman, 1994). In a one-generation reproductive/developmental toxicity study (Bio/dynamics Inc., 1983), male and female CD rats were exposed to 0, 60, 250, or 500 ppm xylenes (technical grade xylene: 2.4% toluene, 12.8% ethylbenzene, 20.3% p-xylene, 44.2% m-xylene, 20.4% o-xylene) by inhalation for 6 hours per day, 5 days per week, for 131 days prior to mating, with exposure continued in the females during gestation days 1-20 and lactation days 5-20. Two additional 500-ppm groups used the same exposure protocol, except that only the F_0 males were exposed in one, and only the F_0 females were exposed in the other. The highest exposure level in this study, 500 ppm, was a NOAEL for reproductive performance in the parental generation. Likewise, a study of male Sprague-Dawley rats exposed to 0 or 1000 ppm xylene solvent for 18 hours per day, 7 days per week, for 61 days reported no differences between control and exposed rats in several testicular endpoints and fertility (Nylén and Hagman, 1994).

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

(Carpenter et al., 1975; Jenkins et al., 1970; Ungváry, 1990). Similarly, high-level exposure to xylenes has resulted in changes in liver morphology, weight, and enzymatic functions (Tátrai and Ungváry, 1980; Tátrai et al., 1981; Ungváry, 1990). Gestational exposure of animals to xylenes has resulted in neurodevelopmental effects (Hass et al., 1995, 1997; Hass and Jakobsen, 1993) and other possible developmental effects (Ungváry et al., 1980; Ungváry and Tátrai, 1985), but only at levels above those at which neurobehavioral effects in adult male rats were reported. Finally, no reproductive effects were found in a one-generation reproductive/developmental study of male and female rats exposed to 500 ppm xylenes (Bio/dynamics, Inc., 1983) or in male rats exposed to 1000 ppm xylenes for 61 days (Nylén and Hagman, 1994).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__I.B.5. Confidence in the Inhalation RfC

Study — Medium
Database — Medium
RfC — Medium

Confidence in the principal study is medium, because the study was an examination of a critical effect of xylenes toxicity that also examined organ weights, body weights, and hematological parameters but was of subchronic duration, examined only one sex of a single species, and did not conduct histologic examination of the animals. Confidence in the database is medium; the database contains several subchronic studies as well as several developmental studies, developmental neurotoxocity studies, and a one-generation reproductive toxicity study. However, a two-generation reproduction study and chronic animal data are lacking. Medium confidence in the RfC results.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF)

__I.B.6. EPA Documentation and Review of the Inhalation RfC

Source Document - U.S. EPA, 2002

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 2002. ***To review this appendix, exit to the toxicological review, Appendix A, Summary of and Response to External Peer Review Comments (PDF)***

Agency Consensus Date - 01/30/2003

__I.B.7. EPA Contacts (Inhalation RfC)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (fax), or hotline.iris@epa.gov (Internet address).

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_VI. Bibliography

Substance Name — Xylenes
CASRN — 1330-20-7
Last Revised — 02/21/2003

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APPENDIX B: DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC ORAL p-RfD

Description of Model-Fitting Procedure for Continuous Data

The model-fitting procedure for continuous data is as follows. When a biologically defined BMR is not available, the default BMR of 1 standard deviation from the control mean response is used (U.S. EPA, 2000). The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated. If the linear model adequately fits the means ($p \geq 0.1$), then it is selected as the model for BMD derivation. If the linear model does not adequately fit the means, then the more complex models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated. If the linear model does not provide adequate fit to the means while the nonhomogenous variance model is applied, then the polynomial, power, and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the dataset is considered unsuitable for modeling.

Modeling of Data on Terminal Body Weight in Male Rats (NTP, 1986)

Following the above procedure, continuous-variable models in the U.S. EPA BMDS (version 1.4.1c) were fit to the data shown in Table B-1 (below) for decreased body weight in male rats exposed for 13 weeks (NTP, 1986) using a biologically based BMR of 10% decrease from the control mean. The constant variance model did not provide adequate fit to the variance data. Further, the variance model included in the BMDS did not provide an adequate fit to the variance, as shown in Table B-2. Because body weight was significantly different from control only in the high-dose group, no attempt was made to exclude this group and model the lower-dose groups. Thus, this dataset was not suitable for BMD analysis.

Table B-1. Final Body Weights of Male Rats Exposed Orally to Mixed Xylenes for 13 Weeks[a]

Dose (mg/kg-day)	Adjusted Dose[b] (mg/kg-day)	Number of Rats	Mean Final Body Weight (g)	Standard Deviation
0	0	10	328	15.8
62.5	44.6	10	323	12.6
125	89.3	10	327	25.3
250	179	10	315	28.5
500	357	10	330	28.5

1000	714	10	291	22.1

[a]NTP, 1986
[b]Adjusted for continuous exposure (adjusted dose = dose × 5/7 days/week)

Table B-2. Model Predictions for Decreased Body Weight in Male Rats Exposed Orally to Xylenes for 13 Weeks[a]

Model	Variance *p*-value [b]	Means *p*-value [b]	$BMD_{0.1}$ (mg/kg-day)	$BMDL_{0.1}$ (mg/kg-day)
All dose groups				
Linear (constant variance) [c]	0.08666	0.11	745.97	517.78
Linear (modeled variance) [c]	0.04729	0.208	849.17	532.93

[a]NTP, 1986
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria (U.S. EPA, 2000)
[c]Coefficients restricted to be negative

Modeling of Data on Terminal Body Weight in male rats (Wolfe, 1988a)

Following the above procedure, continuous-variable models in the U.S. EPA BMDS (version 1.4.1c) were fit to the data shown in Table B-3 (below) for decreased body weight in male rats exposed for 13 weeks (Wolfe, 1988a) using a biologically-based BMR of 10% decrease from the control mean. Using these data, the linear model with constant variance model provided adequate fit to both the variance and means data, as shown in Table B-4. Figure B-1 shows the fit of the linear model with constant variance to the data.

Table B-3. Final Body Weights of Male Rats Exposed Orally to Mixed Xylenes for 13 Weeks[a]

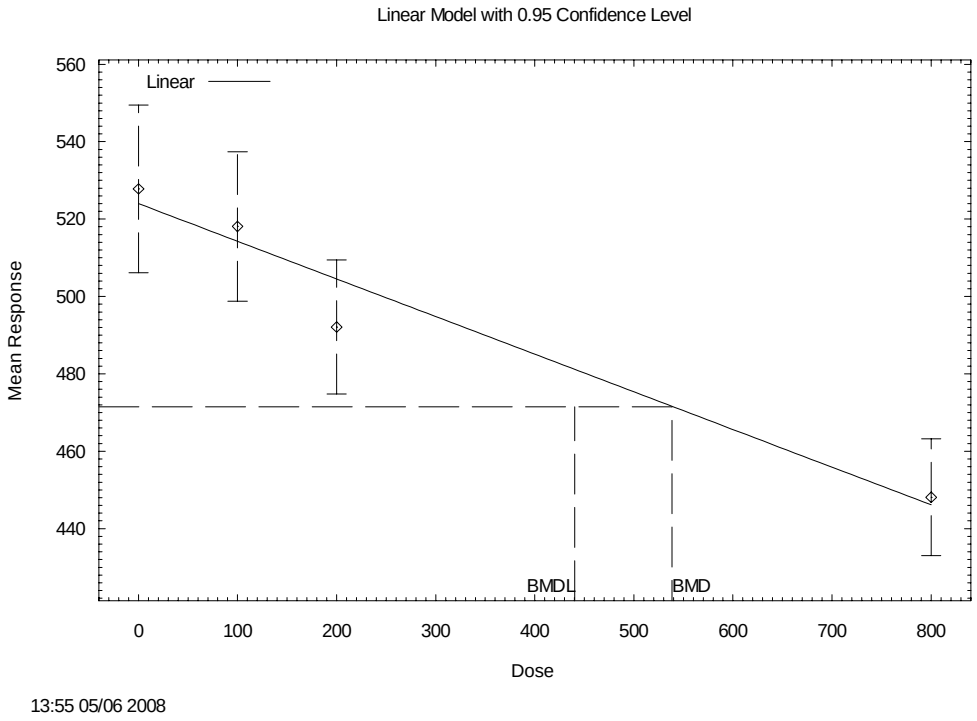
Dose (mg/kg-day)	Number of Rats	Mean Final Body Weight (g)	Standard Deviation
0	20	527.8	46.28
100	17	518.1	37.55
200	15	492.1	31.26
800	18	448.1	30.37

[a]Wolfe, 1988a

Table B-4. Model Predictions for Decreased Body Weight in Male Rats Exposed Orally to Xylenes for 13 Weeks[a]

Model	Variance *p*-value[b]	Means *p*-value[b]	$BMD_{0.1}$ (mg/kg-day)	$BMDL_{0.1}$ (mg/kg-day)
All dose groups				
Linear (constant variance)[c]	0.2173	0.3367	538.39	440.22

[a]Wolfe, 1988a
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria (U.S. EPA, 2000)
[c]Coefficients restricted to be negative



BMDs and BMDLs indicated are associated with a 10% decrease from control body weight and are in units of mg/kg-day.

Figure B-1. Fit of Linear Model (Constant Variance) to Data on Final Body Weight in Male Rats (Wolfe, 1988a)

APPENDIX C: DESCRIPTION OF LITERATURE SEARCH PROCESS FOR XYLENE

The IRIS Toxicological Review (U.S. EPA, 2003) contained a thorough review of oral toxicity data on xylene, so searches were limited to studies published since 2002. The search for additional studies of xylene included terms to identify human exposure studies (epidemiologic, occupational) and animal studies for all relevant noncancer endpoints. The search included health effects and toxicity information available from the U.S. EPA (IRIS), ATSDR and other relevant federal, state or international governmental or quasi-governmental agencies, including, but not limited to ACGIH, NIOSH, OSHA, NTP, IARC, WHO, and CalEPA. In addition, electronic databases, including: CURRENT CONTENTS, MEDLINE, TOXLINE, BIOSIS/TOXCENTER, TSCATS/TSCATS2, CCRIS, DART/ETIC, GENETOX, HSDB, and RTECS, were searched. An electronic listing of all results of the gross literature review (including titles, references and abstracts) and a tabular summary of the search results were provided to EPA.

A toxicologist screened the literature searches based on review of abstracts and titles for studies pertaining to the health effects from subchronic oral exposure to xylenes in humans and animals. Decisions about whether to further consider a particular citation were based on the scientific judgment of the toxicologist, based on reading the abstract provided in the literature search output. Studies that were not considered pertinent were not retrieved. Citations may also have been excluded after retrieval and review of the article by the toxicologist. A study may have been excluded if its scope was outside the scope of the use under consideration, if it was not relevant or appropriate, if its study design was inadequate, or if the study showed inadequacy of quality control or flaws in the interpretation of results.

No new pertinent studies were identified.