U.S. Army Public Health Center

Wildlife Toxicity Assessment for Mixed Xylenes

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Mark A. Williams, Ph.D., FAAAAI
Joseph P. Sullivan, Ph.D.
Gunda Reddy Ph.D., DABT

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U.S. Army Public Health Center
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8252 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

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Xylene, also known as dimethyl benzene or simply referred to as “mixed xylenes,” is a chemical mixture appearing as an aromatic hydrocarbon that exists in three isomeric forms: ortho-, meta- and para-xylene (or 2-, 3-, and 4-xylene). This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of mixed xylenes on wildlife. Evaluating the toxicity of mixed xylenes will contribute to the derivation of toxicity reference values (TRVs) for use as screening-level benchmarks for wildlife near contaminated sites. The protocol for the performance of this WTA is available in detail in Technical Guide No. 254 (Standard Practice for Wildlife Toxicity Reference Values).

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Key Technical Authors: Mark A. Williams, Ph.D., FAAAAI
Toxicology Directorate, Health Effects Division
U.S. Army Public Health Center (APHC)

Joseph P. Sullivan, Ph.D.,
Ardea Consulting, Woodland, CA

Contributing Authors: Gunda Reddy, Ph.D., DABT
Toxicology Directorate, Health Effects Division
U.S. Army Public Health Center (APHC)

External Reviewers: Anonymous: Coordinated via the Society of Environmental Toxicology and Chemistry

Point of Contact
For further information or assistance, please contact the primary author:

Mark A. Williams Ph.D., FAAAAI
U.S. Army Public Health Center
Toxicology Directorate, Health Effects Division
ATTN: MCHB-PH-TOX; Building E2100
8252 Blackhawk Road, Aberdeen Proving Ground MD 21010-5403
(410) 436-3980/DSN 584-3980
Email: usarmy.apg.medcom-phc.mbx.tox-info@mail.mil

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Department of the Army
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Wildlife Toxicity Assessment for Mixed Xylenes

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1. INTRODUCTION

Xylene, also known as dimethyl benzene or simply referred to as “mixed xylenes,” is a chemical mixture appearing as an aromatic hydrocarbon that exists in three isomeric forms: *ortho-, meta*- and *para*-xylene (or 2-, 3-, and 4-xylene). A mixture of the xylene isomers is frequently called xylol. Depending on whether derived from petroleum or coal tar sources, mixed xylenes may have varying ratios of the three-xylene isomers. Ethylbenzene is isomeric with the xylenes (same molecular formula), has similar physicochemical properties, and is usually present as a significant contaminant of mixed xylenes (Low et al. 1989; WHO 1997).

Mixed xylenes from petroleum sources contain approximately 20% *ortho*-xylene, 44% *meta*-xylene, 20% *para*-xylene, and 15% ethylbenzene. By contrast, mixed xylenes from coal tar are comprised of approximately 10-15% *ortho*-xylene, 45-70% *meta*-xylene, 23% *para*-xylene, and 6-10% ethylbenzene (NIOSH 1975; Meek and Chan 1994). Small quantities of toluene and C9 aromatic fractions may also be present (Fishbein 1988 in WHO 1997). Since the different xylene isomers and ethylbenzene have similar properties, it is generally not economical to separate them (Low et al. 1989; WHO 1997). Commercial xylene might also contain low levels of toluene, trim ethylbenzene, phenol, thiophene, and pyridine and non-aromatic hydrocarbons. In addition, commercially available xylene is frequently contaminated with benzene (WHO 1997).

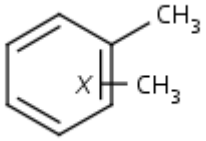
The xylenes mixture, at room temperature, is a colorless liquid with an aromatic sweet odor and a moderate solubility in water. Approximately 92% of mixed xylenes are blended into gasoline. It is also used in a variety of solvent applications, particularly in the paint and printing ink industries and is found in coal tar and natural gas. The single largest end-use of mixed xylenes is synthesis of the *para*-xylene isomer. The major derivatives synthesized from *para*-xylene are dimethyl terephthalate and terephthalic acid that are used in the manufacture of polyester fiber, film and fabricated items. Mixed xylenes are also used in the manufacture of perfumes, pesticide formulations, pharmaceuticals and adhesives, and in the painting, printing, rubber, plastics, and leather industries (Low et al. 1989; Meek and Chan 1994; WHO 1997; Fay et al. 1998). In addition, mixed xylenes are used as degreasing and cleaning agent, and coating and impregnating fabrics and paper products.

Mixed xylenes also occur naturally in some wild growing plants and emitted from agricultural crops including alfalfa, corn, and cereal silage. Xylenes are also emitted from burning wood products in fireplaces and heating stoves, and during times of ecologically deliberate and inadvertent wildfires. Given their widespread use, it is not surprising that they are found as contaminants at hazardous waste sites (Low et al. 1989; Fay et al. 1998). In addition,

commercial production and use of xylene as a solvent and chemical intermediate might result in its release to the environment through various waste streams (HSDB 2019). The majority of xylene released into the environment enters the atmosphere directly where it is broken down, although it is not degraded by exposure to sunlight (WHO 1997). Its use as an herbicide will result in its direct release to the environment. Common and naturally occurring sources of mixed xylenes are petroleum, inadvertent wildfires, and volatiles that are derived from plants. The processing and use of petroleum products, in addition to leaks and evaporation losses during the transport and storage of gasoline and other fuels might result in the release of xylenes into the environment (HSDB 2019).

This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of mixed xylenes on wildlife. Evaluating the toxicity of mixed xylenes will contribute to the derivation of toxicity reference values (TRVs) for use as screening-level benchmarks for wildlife near contaminated sites. The protocol for the performance of this WTA is available in detail in Technical Guide No. 254 (*Standard Practice for Wildlife Toxicity Reference Values)* (USACHPPM 2000) and previously published resources (Johnson and McAtee 2015; Deck and Johnson 2015).

Table 1. Summary of the Physical-Chemical Properties of Xylenes

Structure	
CAS No.[1]	1330-20-7
Molecular weight[1]	106.17
Color[1]	Colorless
Physical state[1]	Liquid and mobile
Melting point[1]	-25.182°C (*ortho*-xylene) -47.872°C (*meta*-xylene) 33.263°C (*para*-xylene)
Boiling point (760 mmHg)[4]	138.5°C (mixed xylene)
Odor[1]	Characteristic sweet odor
Solubility in water[4]	130 – 190 mg/L
Solubility in other solvents[1]	Completely miscible in absolute alcohol, ethyl ether, and many other organic liquid solvents
Partition coefficients:	
Log K_{ow}[1]	3.15
Log K_{oc}[2]	Range from 1.59 – 2.56
Vapor pressure at 25°C[4]	5 to 9 mm Hg
Henry's Law constant at 25°C	5.18×10^{-3} to 7.18×10^{-3} atm.m^3/mole[2] 6.4×10^{-3} atm.m^3/mole[4]
Vapor density[3]	3.7
Conversion factors[1, 5]	Concentration in air: 1 mg/m^3 = 0.227 ppm (V/V); 1 ppm (V/V) = 4.41 mg/m^3; 1 ppm = 4.35 mg/m^3 at 25°C, 101.3 kPa[5] 1 mg/m^3 = 0.23 ppm at 25°C, 101.3 kPa[5]

Legend:
°C = degrees Celsius
mg/L = milligrams per liter
Log Koc = octanol-water partition coefficient
Log Koc = organic carbon partition coefficient
mm Hg = millimeters of mercury
atm.m^3/mole = cubic meters of atmosphere per mole
mg/m^3 = milligrams per cubic meter
mm Hg = millimeters of mercury
kPa = kilopascal

Table 1 Notes (continued):
Source:
[1]Jori et al. 1986
[2]HSDB 2019
[3]Low et al. 1989
[4]U.S. EPA 2005a
[5]WHO 1997

2. TOXICITY PROFILE

2.1 Literature Review

Electronic searches of relevant biomedical, toxicological, and ecological databases (*e.g.*, PubMed®, BIOSIS®, Defense Technical Information Center (DTIC) On-Line Multisearch, Scopus, Web of Science and TOXNET® – an aggregated tool for simultaneously searching the following databases: HSDB® [Hazardous Substances Data Bank]; TOXLINE®; ChemIDplus®; IRIS [Integrated Risk Information System]; LactMed®; DART® [Developmental and Reproductive Toxicology]; TOXMAP®; TRI [Toxic Release Inventory]; CTD [Comparative Toxicogenomics Database]; Household Products Database; Haz-Map®; ITER [International Toxicity Estimates of Risk]; ALTBIB [Alternative to Animal Testing Bibliography]; CCRIS [Chemical Carcinogenesis Research Information System]; CPDB [Carcinogenic Potency Database]; and GENE-TOX were conducted on September 9–11, 2010 and were updated again on September 19 and 21, 2012, June 25–27, 2018, and August 8–9, 2019, with the aim of identifying primary reported studies and reviews on the toxicology of mixed xylenes. Searches were revised and updated on August 8–9, 2019. Separate searches were conducted for general toxicology and specific searches for birds, reptiles, amphibians, and wildlife. Each database was searched using key words such as xylene or its Chemical Abstract Service (CAS) number (No.) (1330-20-7) plus toxicity, ecotoxicology, wild, wildlife, avian, bird, frog, amphibian, reptile, or environment. Appendix A documents the details and results of the search strategies. The titles of articles identified in each search were reviewed for relevance. Potentially relevant articles focused on the toxicological effects of mixed xylenes on terrestrial vertebrates or its environmental fate and transport. All potentially relevant articles were acquired as electronic files or by visiting the University of California, Davis libraries and the Johns Hopkins University School of Medicine's libraries. Review articles provided additional articles not identified during the initial database searches.

2.2 Environmental Fate and Transport

Little information exists regarding the amount of xylene in surface water and soil. However, levels of xylene in contaminated groundwater have been reported to be as high as 10000 parts per billion (ppb) (ATSDR 2007). The environmental fate, transport, partitioning, transformation, and degradation of xylene are expected to be similar for each of the component isomers based on the similarities of their physical and chemical properties.

Xylene is sometimes released into the water and soil as a result of the use, storage, and transport of petroleum products. For example, notable accidental spills of xylene to the Mississippi River waterways and the Zhuhai Port in China caused temporary closure of large

sections of those waterways and marked increases in the concentrations of xylene present in those water columns that created a threat to not only drinking water quality but also to aquatic life (Duan et al. 2017). In addition, xylene was recognized as being among the top 100 chemicals, and the Health and Safety Representatives (HASREP) project recognized xylene as one of the 15 bulk hazardous and noxious substances (HNSs) transported and dealt with across European waterways (Duan et al. 2017). In addition, xylene is on a list of the top 20 chemicals likely to present the highest risk of being involved in an HNS incident as recorded by the International Maritime Organization or IMO (Duan et al. 2017).

With regards to xylene surface water concentrations found across several geographic regions of the world, it was found that the levels were far less than the 500 micrograms per liter (µg/L) World Health Organization's (WHO) water quality standard, (WHO Water Quality Criteria and Environmental quality standards for surface water for xylene; SEPA 2002; PAN 2017 both as cited in Duan et al. 2017). However, the concern from accidental xylene spillages to water systems is the significant risk of harm to marine and aquatic ecosystems (Duan et al. 2017). Consensus opinions are that the environmental behavior of mixed xylenes (or indeed its constituent isomers) places it in the floater/evaporate (FE) category (ATSDR 2007). This behavior is largely due to a combination of its vapor pressure of 5–9 millimeters of mercury (mmHg) at 25°C, a density of 0.861–0.880 gram per cubic centimeter (g/cm^3) and a solubility of between 130–190 milligrams per liter (mg/L) of water (Duan et al. 2017).

Volatilization to the atmosphere from water is rapid for all three isomers because of their high vapor pressure and low water solubility; this pathway is considered the primary removal pathway for xylenes from surface water (in addition to biodegradation pathways). There is no reported information on the volatilization rate of xylenes from soils, but it is expected to be a rapid process, at least from near to the surface because of the high volatility of xylenes. In the atmosphere, the xylene isomers are readily degraded, primarily by photo-oxidation. In soil and water, the *meta*- and *para*- isomers are readily biodegraded under a wide range of aerobic and anaerobic conditions, but the *ortho*- isomer is more persistent. Wilson-Durant *et al.* (1999) demonstrated that xylene degrades as the result of biological action under aerobic conditions, but not as readily under anaerobic conditions. When mixed xylenes are degraded under anaerobic conditions by a microbial culture derived from xylene contaminated groundwater, *meta*- and *para*-xylene were fully degraded within 8 days; however, *ortho*-xylene degraded much more slowly and degradation ceased once the other substrates had been consumed (Szykowny and Keasling 1997). In soil under aerobic conditions, *ortho*- and *para*-xylene were metabolized at a faster rate when benzene and toluene were present than when they were incubated alone (Tsao et al. 1998). *Meta*-xylene did not biodegrade under anaerobic conditions but was extensively biodegraded (up to 89%) after 83 days when oxygen was present (Wilson-Durant et al. 1999).

The white-rot fungus, *Phanerochaete chrysosporium* will degrade each of the xylene isomers. When evaluated separately over a 7-day period, the degradation of *meta*- and *para*-xylene was comparable, but the degradation of *ortho*-xylene was slightly higher. The rate of degradation was higher at 25°C than at 37°C (Yadav and Reddy 1993).

Xylenes are likely to be adsorbed to a small extent onto both aquatic sediments and soil, based on their partition coefficients. However, adsorption is dependent on such factors as the organic carbon content and the water content (WHO 1997).

2.3 Bioaccumulation and Elimination

Bioaccumulation of xylene isomers by fish and invertebrates is likely limited. Elimination of xylene from aquatic organisms is rapid once exposure has ceased (WHO 1997). Reported bioconcentration factors (BCFs) suggest that xylenes are of minimal concern with regard to their potential for bioaccumulation in aquatic species (Beek et al. 2000, as discussed in Duan et al. 2017). It is generally appreciated that BCF values of less than 20 are of little or no bioconcentration risk, a BCF of 100 indicates a low potential for accumulation, and at 100–1000 BCFs are considered to be of moderate risk. Should BCFs exceed 1000, then this value would be considered high risk. In addition, bioconcentration has been predicted for all isomers of xylene since they tend to partition into the octanol phase of the octanol-water system. It has since been found that BCF values for *meta-, ortho*-, and *para*-xylene were respectively 251, 218, and 257 in the green algae *Selenastrum capricornutum* (Herman et al. 1991). In addition, BCF values of 21.4–23.6 that were found for eels *(Anguilla japonica)* also indicated that bioconcentrations of mixed xylenes are unlikely (Ogata and Miyake 1978).

The rapid oxidation of xylene isomers to their corresponding polar metabolites would likely preclude bioconcentration in higher animal systems up the food chain and, thus, bioaccumulation of the food chain is unlikely (U.S. EPA 1985b). The BCFs for *meta*-, *ortho*- and *para*-xylenes were estimated as 45, 105, and 95 respectively (U.S. EPA 1985b).

Like all classes of aromatic hydrocarbons, xylene is expected to be highly lipid soluble and, thus, readily absorbed from the gastrointestinal track of mammals. In mammalian species like the rat for example, it was found that following exposure to 47.8-ppm *para*-xylene vapors, xylene appeared predominantly in the kidneys (up to approximately 1000 nanomole per gram (nmol/g) tissue or 0.11 milligrams per gram (mg/g)) as compared to the next highest tissue, and subcutaneous fat, at less than 300 nmol/g tissue (0.032 mg/g). Blood concentrations quickly reach steady state and remain nearly unchanged following a 1 or an 8 hour exposure. Concentrations diminish quickly in all tissues other than in fat and ischiadic nerve tissue once exposure is terminated. In other tissues, concentrations diminish to about 50% of their prior concentrations after as little as 1 hour. The half-life of xylene in fat was approximately 2.2 hours after 1 hour of exposure and 6.9 hours after 8 hours of exposure (Carlsson 1981).

2.4 Summary of Mammalian Toxicity

2.4.1 Mammalian Oral Toxicity

2.4.1.1 Mammalian Oral Toxicity—Acute

The acute oral LD_{50} in rats was reported as 10.0 milliliters per kilogram (mL/kg) (8600 milligrams per kilogram (mg/kg)) (Hine and Zuidema 1970 in Low et al. 1989), 3287 mg/kg (Procter and Gamble Company 1978), 3500 mg/kg (Younger Laboratories 1978), 3523 mg/kg (NTP 1986),

4300 mg/kg (Wolf et al. 1956), and between 3523 and 8700 mg/kg (U.S. EPA 2005a). The LD_{50} [lethal dose 50%] value of 3287 mg/kg (Procter and Gamble Company 1978) was less than the lowest reported dose of 3300 mg/kg, so this reported value is suspect. In the study by Muralidhara and Krishnakumari (1980), a dose of 5950 mg/kg administered to female rats was not only the calculated LD_{50} but also the minimal lethal dose.

NTP (1986) reports lack of coordination, prostration, loss of hind leg movement, and hunched posture within 24 hours of dosing in male and female rats that received 4000 or 6000 mg/kg. Male and female rats receiving 2000 mg/kg had rough coats. No clinical signs of toxicity were noted in the surviving animals at the end of week 1. Body weight gain was decreased in the group receiving 4000 mg/kg (NTP 1986). Other clinical signs included weight loss, increasing weakness, depression, loss of placement, righting and stretch reflex, lacrimation, hyperpnoea, tremors, gasping, coma, and collapse. Necropsies found lung and liver hyperemia, mottled liver, enlarged, discolored, and hemorrhagic kidneys, discolored spleen, hemorrhagic adrenals with loss of demarcation between medulla and cortex, gastrointestinal inflammation (Procter and Gamble Company 1978; Younger Laboratories 1978).

Muralidhara and Krishnakumari (1980) dosed female rats with 4, 5, 6, 7, 8, 10, and 12 milliliters per kilogram (mL/kg) (3400, 4250, 5100, 5950, 6800, 8500, and 10200 mg/kg) and determined the minimum lethal dose was 5950 mg/kg with a no-observed-adverse-effect level (NOAEL) of 5160 mg/kg-day. Symptoms manifested at fatal doses of 5950 mg/kg and higher were an immediate state sluggishness, appearing within 4–6 hours, dullness, stupor, anesthesia, narcosis, and coma. Early deaths occurred within 24 hours and maximum mortality occurred within 72 hours (Muralidhara and Krishnakumari 1980).

In mice, the LD_{50} was reported to be 5627 mg/kg in male mice and 5251 mg/kg in female mice. In mice, tremors, prostration, and/or slowed breathing were observed within 48 hours of dosing with 4000 or 6000 mg/kg. No differences were noted for body weight changes (NTP 1986).

Table 2. Summary of Acute Oral Toxicity for Mixed Xylenes in Mammals

Test Organism	LD_{50} (mg/kg)	Test Results			
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	Study
Rats	8600	NA	NA	NA	Hine and Zuidema 1970
Rats	NA	5100	5950	Sluggishness, dullness, stupor, anesthesia, narcosis and coma	Muralidhara and Krishnakumari 1980
Rats	NA	<3300	3300	Clinical signs: depression, loss of placement, righting and stretch reflex, lacrimation, hyperpnea, tremors, gasping, coma and death; necropsy: gastrointestinal irritation, hemorrhagic kidneys and adrenals with loss of demarcation between medulla and cortex in deaths; in survivors showed mottled liver, irritated gastrointestinal tract, dark spleen, enlarged kidneys	Proctor and Gamble Company 1978
Rats	3500	2510	3160	Clinical signs: weight loss, increasing weakness, collapse and death; necropsy: lung and liver hyperemia, discoloration of kidneys and spleen, gastrointestinal inflammation	Younger Laboratories 1978
Rats	3523	2000	4000	Lack of coordination, prostration, loss of hind leg movement, and hunched posture, body weight gain was decreased	NTP 1986
Rats	4300	NA	NA	NA	Wolf et al. 1956
Rats	3523 - 8700	NA	NA	NA	U.S. EPA 2005a
Mice (male) Mice (female)	5627 5251	2000	4000	Tremors, prostration, and/or slowed breathing	NTP 1986

Legend:
LD_{50} = Lethal Dose 50%
mg/kg = milligrams per kilograms
NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
NA = not applicable

2.4.1.2 Mammalian Oral Toxicity—Subchronic

Condie et al. (1988) dosed Sprague Dawley rats with mixed xylenes (*ortho*-xylene, 17.6%; *meta*- and *para*-xylene, which co-eluted, 62.3% and ethyl benzene, 20.0%) for 90 days. Increased absolute and relative liver weights occurred in male rats dosed with as little as 150 mg/kg-day. Female rats dosed with 750 mg/kg-day exhibited increased absolute and relative liver weights. The only clinical observation during this study was an increased aggressiveness in male rats receiving 1500 mg/kg-day. Additional adverse effects in males were increased absolute and relative kidney weights in rats dosed with 750 or 1500 mg/kg-day; decreased average body weights and heart weight in males dosed with 1500 mg/kg-day; increased hematocrit and platelets in males dosed with 1500 mg/kg-day; increased corpuscular hemoglobin in males dosed with 750 and 1500 mg/kg-day.

Additional adverse effects in females were increased absolute and relative heart and spleen weights females dosed with 1500 mg/kg-day; increased absolute and relative kidney weights in females dosed with 1500 mg/kg-day; increased red blood cell count, hemoglobin, and hematocrit in females dosed with 1500 mg/kg; elevated white blood cell count in females dosed with 750 and 1500 mg/kg-day. Histological changes in kidneys of both male and females indicated early stages of chronic nephropathology, mostly at 750 and 1500 mg/kg-day. For males, no NOAEL values were identified. The LOAEL was 150 mg/kg-day in males based on absolute and relative liver weights. In females, the NOAEL was 150 mg/kg-day. The LOAEL in females was 750 mg/kg-day based on absolute and relative liver weights and elevated white blood cell count.

In a 28-day feeding study (Procter and Gamble 1978), no albino rats died when exposed to up to 1098 mg/kg-day. No gross signs of toxicity were noted and at necropsy, a slight incidence of pale and enlarged kidneys, and mottled livers in all experimental groups.

When mice were dosed from gestation day (GD) 6–15 with 0.6, 1.2, 2.4, 3.0, 3.6, or 4.2 mL/kg/day (equivalent to 516, 1032, 2064, 2580, 3096, and 4128 mg/kg-day), a dose of 4128 mg/kg/day killed all 15 dams. A dose of 3096 mg/kg/day killed 12 of 38 and caused a significant decrease in weight gain. Lower doses did not have effect on maternal weight gain or cause mortality. Doses of 2064 and 2580 mg/kg/day caused significant increase in maternal liver weight. These results indicated 2580 mg/kg/day was maximum tolerated dose (RTI 1979; Marks et al. 1982).

In a 14-day toxicity test (NTP 1986), rats were dosed a single time daily for 14-consecutive days via gavage with 125, 250, 500, 1000, 2000 mg/kg. Xylenes caused 3/5 deaths in male rats and 4/5 female rats deaths at 2000 mg/kg. No other treatment-related mortality occurred. The change in mean body weight relative in treated rats to that of controls was 23-29% lower for males dosed with 250, 500, and 1000 mg/kg and 17% and 26% lower for females that received 125 and 1000 mg/kg after 14 days. Shallow, labored breathing and prostration were observed immediately after dosing for male and female rats that received 2000 mg/kg.

In another 14-day toxicity test (NTP 1986), mice were dosed a single time daily for 14-consecutive days via gavage with 250, 500, 1000, 2000, and 4000 mg/kg. Mortality was 5/5

male mice and 5/5 female mice at 4000 mg/kg. No other treatment related mortality occurred. Male mice that received 2000 mg/kg gained notably less weight than did the controls. Female mice that received 2000 mg/kg gained more weight than did the controls. During week 1, prostration and shallow breathing were observed after dosing in mice that received 2000 mg/kg.

In a 13-week toxicity bioassay (NTP 1986), rats were dosed with 62.5, 125, 250, 500, and 1000 mg/kg 5 days a week via oral gavage. Xylenes caused 0/10 male and 0/10 female deaths at 1000 mg/kg. No treatment-related mortality occurred, and all animals survived to the end of the study. Changes in mean body weight of male and female rats that received 1000 mg/kg was 15% and 8% lower than that of the controls after 13 weeks of exposure. Evidence of toxicity was not seen, and compound-related gross or microscopic pathologic lesions were absent.

In a second 13-week toxicity bioassay (NTP 1986), mice were dosed with 125, 250, 500, 1000, and 2000 mg/kg for 5 days a week via oral gavage. This study showed that 0/10 male and 2/10 female deaths were found at a dose of 2000 mg/kg in mice. No additional treatment-related mortality occurred in mice. Weakness, lethargy, short and shallow breathing, unsteadiness, tremors, and paresis were observed in the 2000 mg/kg group at approximately 5–10 minutes after dosing, which lasted for approximately 15–60 minutes. Mean body weight gain of mice that received 2000 mg/kg was 7% lower than that of the vehicle controls for males and 17% lower for females. No compound-related gross or microscopic pathological lesions were reported.

Table 3. Summary of Subchronic Oral Toxicity for Mixed Xylenes in Mammals

Test Organism	Test Duration	Test Results			
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	Study
Albino rats	28 days	1098	>1098	No effects at highest dietary level	Proctor and Gamble Company 1978
Sprague-Dawley rats	90	Males: <150 Females: 150	150 750	Increased absolute and relative liver weights in males and females. In females, elevated white blood cell counts and chronic nephropathology	Condie et al. 1988
CD-1 mice	GD 6 - 15	1032	2064	Significant increase maternal liver weight	RTI 1979, Marks *et al.* 1982
F344/N rats	14 days	Males: 125 Females: <125	250 125	Decreased body weight change	NTP 1986
B6C3F1 mice	14 days	Males: 1000 Females:1000	2000 2000	Changes in body weight gains and prostration and shallow breathing	NTP 1986
F344/N rats	13 weeks	Males: 500 Females: 500	1000 1000	Reduced body weights	NTP 1986
B6C3F1 mice	13 weeks	Males: 1000 Females: 1000	2000 2000	Weakness, lethargy, short and shallow breathing, unsteadiness, tremors, and paresis, and reduced body weights	NTP 1986

Legend: mg/kg = milligrams per kilograms; NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level; CD = cluster of differentiation

2.4.1.3 Mammalian Oral Toxicity—Subchronic: Developmental Toxicity

Mixed xylenes were teratogenic to cluster of differentiation (CD)-1 mice when dosed via gavage from GD 6–15, with a dose response effect observed as the dose was increased from 0.6 to 3.6 mL/kg/day (equivalent to 516 to 3096 mg/kg-day). A dose of 3096 mg/kg-day caused a significant increase in resorptions. Fetal weights decreased at doses of 2064, 2580, and 3096 mg/kg-day, but the numbers of stunted fetuses at the same doses was not increased. The numbers of implants was not affected. The percent of resorptions increased at 3096 mg/kg/day only. Doses of 2064, 2580, and 3096 mg/kg/day caused an increase in number of fetuses with external malformations. External malformations included cleft palate, bilateral open eye, and exencephaly. No changes in the numbers of animals presenting with visceral or skeletal malformations were observed. Wavy ribs, in most instances bilateral or at least involving several ribs, were found at 2580 and 3096 mg/kg/day (RTI 1979; Marks et al. 1982).

2.4.1.4 Mammalian Oral Toxicity—Chronic

In a 2-year chronic study in rats where doses were 0, 250, or 500 mg/kg/day, mortality in males was: 11/50 (control), 16/50 (250 mg/kg/day), and 19/50 (500 mg/kg/day); and in females this was: 11/50 (control), 15/50 (250 mg/kg/day), and 13/50 (500 mg/kg/day). Survival of the high dose group of male rats was significantly lower than the controls after week 103 (NTP 1986). In a similar study with mice receiving doses of 0, 500, and 1000 mg/kg/day, mortality in males was: 19/50 (control), 15/50 (500 mg/kg/day), and 11/50 (1000 mg/kg/day); and in females: 14/50 (control), 14/50 (500 mg/kg/day), and 19/50 (1000 mg/kg/day). Hyperactivity occurred in all high dose (1,000 mg/kg/day) treated male and female mice at approximately 5–30 minutes after dosing and was observed consistently during weeks 4–103 of the studies (NTP 1986).

2.4.1.5 Mammalian Inhalation Toxicity—Acute

The lethal concentration 50 (LC_{50}) via inhalation in rats that were exposed to mixed xylenes for 4 hours was 6350 parts per million (ppm) (Hine and Zuidema 1970; Low et al. 1989). The acute inhalation LC_{50} in rats is reported to be between 5000 and 10950 ppm for mixed xylenes (U.S. EPA 2005a). The 4-hour inhalation LC_{50} in male rats was estimated nominally as 29 mg/L (6600 ppm) when exposed to mixed xylenes comprised of ethylbenzene- 19%, *para*-xylene- 8%, *meta*-xylene- 65%, and *ortho*-xylene- 8%. Measured concentrations were roughly 60% of the metered rates so the actual LC_{50} is approximately 17 mg/L (3900 ppm).

Surviving rats at metered rates of 26 and 12 mg/L (5900 and 2700 ppm) became prostate but recovered and appeared normal during 14-day observation period. At 5.8 mg/L (1300 ppm), rats became uncoordinated but recovered. No impacts observed in rats exposed to 2.5 mg/L (570 ppm) (Carpenter et al. 1975). No mortality occurred in rats when exposed to 21.1 mg/L (4800 ppm) for 6 hours. Clinical signs included very slight discomfort during hours 0-3; labored breathing, lethargy, roughened fur, increasing weakness, and collapse in three instances during hours 3–6. After exposure, weight loss persisted for 1–2 days (Younger Laboratories 1978). It was found that rats exhibited hearing loss when they were exposed for 8 hours to 1450 ppm via inhalation on a single day or 3 consecutive days (Pryor et al. 1987). Exposure to mixed

xylenes via inhalation to 1800 ppm for 8 hours a day for 5 days caused a hearing deficit from 8 to 24 kilohertz (kHz) (Crofton et al. 1994).

Inhalation exposure to decreasing concentrations of mixed xylenes from 800 through 3000 ppm, for 8 hours per day, for 5-consecutive days provoked increased impairment in the ability of rats to hear auditory stimuli as measured using the brainstem auditory-evoked response (BAER) elicited by 16 kHz tone pips. The use of this procedure for the examination of hearing is based in the fact that as stimulus intensity is decreased, the amplitude of the response also decreases. When separate single exposure tests were conducted, 2000 ppm caused a significant difference to controls, but 1700 ppm did not (Rebert et al. 1995).

Rats exposed to 2000 ppm mixed xylenes (2% of *ortho*-xylene, 64.5% of *meta*-xylene, 10% *para*-xylene, 23% of ethylbenzene, 0.5% of toluene and 4 ppm of benzene) via inhalation for 6 hours a day for 3 days exhibited increase in the liver to body weight ratio, but no increase in the kidney to body weight ratio (Toftgård and Nilsen 1982).

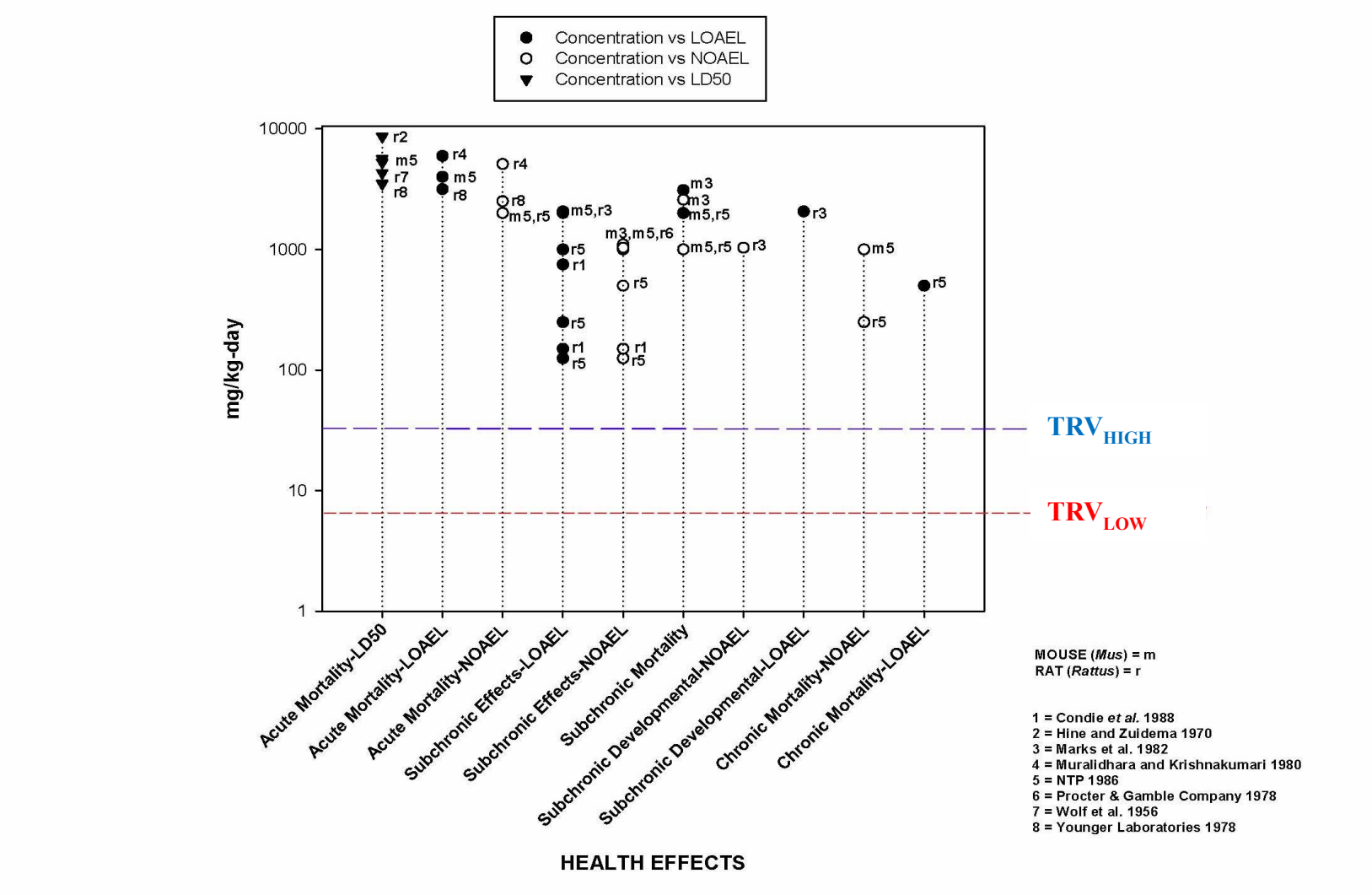


Figure 1. Mixed Xylenes: Oral Ingestion Health Effects to Mammals

Adult male rats were exposed to 300-ppm xylene containing 80% of *meta*-xylene and 12% *para*-xylene for 6 hours a day 5 days a week for 2 weeks. Behavioral changes noted 17 hours following the exposure on the 4th day showed a decrease in ambulation, no change in rearing, notable, but nonsignificant increase in preening frequency and preening time, and no change in defecation or urination frequency. The effects caused by xylene alone were inconspicuous and probably insignificant from toxicological point of view (Savolainen et al*.* 1978).

Table 4. Summary of Acute Inhalation Toxicity for Mixed Xylenes in Mammals

Test Organism	LC_{50} (ppm)	Test Results			
		NOAEL (ppm)	LOAEL (ppm)	Effects Observed at the LOAEC	Study
Rats (4-hr)	6350	NA	NA		Hine and Zuidema 1970
Rats	5000 – 10,950	NA	NA		U.S. EPA 2005a
Rats (4-hr)	3900	570	1300	Uncoordinated	Carpenter et al. 1975
Rats (6-hr)	NA	NA	4800	Partial discomfort reported during hours 0-3; labored breathing, lethargy, roughened fur, increasing weakness, and collapse	Younger Laboratories 1978
Rats (8-hr)	NA	NA	1450	Hearing loss	Pryor et al. 1987
Rats (8-hr for 5 days)	NA	NA	1800	Hearing loss	Crofton et al. 1994
Rats (8-hr for 5 days)	NA	1700	2000	Hearing loss	Rebert et al. 1995
Rats (6-hr for 3 days)	NA	NA	2000	Increased the liver to body weight ratio	Toftgård and Nilsen, 1982

Legend:
LC_{50} = lethal concentration
ppm = part(s) per million
NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
LOAEC = lowest-observable-adverse-effect concentration

Carpenter et al*.* (1975) exposed cats to mixed xylenes that was comprised of ethylbenzene- 19%, *para*-xylene- 8%, *meta*-xylene- 65%, and *ortho*-xylene- 8%. Four cats exposed to a

measured concentration of 41 mg/L (9300 ppm) all died within 2 hours. The response of the animals followed a time-related pattern of salivation, ataxia, tonic and clonic spasms, and anesthesia followed by death. These toxic signs are suggestive of a central nervous system effect. No abnormal histopathological findings were reported.

When groups of cats (five animals/group) were exposed for 5740, 6900, or 9200 ppm for up to 6 hours, there was a concentration-dependent decrease in time to onset of staggering and mild narcosis. However, there was a significant variability seen between individuals. Deep narcosis was also seen in four animals at the expose 9200-ppm xylene (Engelhardt and Estler 1935 in WHO 1997).

2.4.1.6 Mammalian Inhalation Toxicity—Subchronic

Continuous exposure of rats to unspecified mixed xylenes varying from 550 to 750 ppm via inhalation for 21 days did not cause any mortality nor impact body weights or behavior (Battig and Grandjean 1964).

Male rats exposed to 630 ± 170-ppm xylenes (23% ethylbenzene, 2% *ortho*-xylene, 64.5% *meta*-xylene, and 10% *para*-xylene, 0.5% toluene, and 4-ppm benzene) for 14 hours per day for 28-consecutive days provoked a significant increase in liver weight and liver to body weight ratios (Toftgård et al. 1981).

Similar to what was observed with acute exposures, rats exposed to xylenes at 1200 ppm via inhalation for 14 hours a day for 42 days exhibited a reduced ability to detect auditory tones which they had been trained to recognize to avoid an electronic shock. No affect was observed at 800 or 1000 ppm (Pryor et al. 1987).

Table 5. Summary of Subchronic Inhalation Toxicity for Mixed Xylenes in Mammals

		Test Results			
Test Organism	Test Duration	NOAEL (ppm)	LOAEL (ppm)	Effects Observed at the LOAEL	Study
Rats	21 days	750	>750		Battig and Grandjean 1964
Male Sprague-Dawley rats	28 days	<630	630	Significant increases in liver weights and liver to body weight ratios	Toftgård et al. 1981
Weanling male Fischer-344 rats	42 days	<1200	1200	Hearing loss	Pryor et al. 1987
Adult male Sprague-Dawley rats	61 days	<1009	1009	Significantly reduced body weights and slight hearing loss	Nylén and Hagman 1994
Adult female Sprague-Dawley rats	12 weeks	<330	330	Renal toxicity	Kum et al. 2007

Legend:
NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
ppm = part(s) per million

When adult male Sprague-Dawley® rats are exposed via inhalation to 1010-ppm technical xylene for 18 hours a day for 61 consecutive days, no animals had testicular alterations. Testes and spermatogenesis appeared to be normal with a mosaic of tubules in different stages of sperm production, 2 weeks and 10 months after treatment with xylene. All three rats exposed to xylene were fertile (Nylén et al. 1989).

Exposure to 1009-ppm mixed xylenes (comprised of *ortho*-xylene 1.5%, *meta*-xylene 65%, and *para*-xylene 32% with ethylbenzene 2.5%) for 18 hours a day for 61 consecutive days significantly reduced body weights at 2 days post-exposure, but the rats had recovered by 2 months post-exposure. No effect on conduction velocities in compound or motor nerve in the tail or in the amplitude of action potential in the same nerves. The exposure caused a slight loss of auditory sensitivity at 12.5 kHz but not at 3.15, 6.3, or 20.0 kHz at 2 days post-exposure (Nylén and Hagman 1994).

In a study that assessed exposure of rats to 330 ppm for 12 weeks, increases in serum urea, glutathione and malondialdehyde were found—observations that indicated renal toxicity (Kum et

al. 2007). Carpenter et al. (1975) conducted 13-week subchronic studies in which dogs and rats were exposed for 6 hours a day. From this study, no adverse effects were noted in the highest measured exposure of 3.5 mg/L (800 ppm).

Subchronic or subacute exposure in some rats exposed to 3000 milligrams per cubic meter (mg/m^3) of mixed xylenes for 8 hours per day, on 6 days per week for 110–130 days, resulted in paralysis of the hind legs, weight loss, a slight decrease in leukocyte numbers, increases in blood urea, urinary blood, and albumin, and hyperplasia of the bone marrow. There was also evidence of partial congestion of the kidney, liver, heart, adrenal glands, the lungs, and spleen. Cellular desquamation of the glomeruli and necrosis of the convoluted tubules were also reported (WHO-IARC 1989).

Subchronic or subacute studies were conducted in groups of male CFY rats that were exposed to air containing 0, 140, 350, or 920 ppm (i.e., 0, 600, 1500, or 4000 mg/m^3) mixed xylenes (comprising 10% *ortho*-xylene, 50% *meta*-xylene, 20% *para*-xylene, and 20% ethylbenzene) for 8 hours per day, 7 days per week for 6 weeks and then for 5 days per week for 6 months (U.S. EPA 2003). No statistically significant differences in body weights were observed in any of the exposed groups when compared with the control values.

Statistically, significant changes that were observed in exposed groups at 6 months (as compared with control group values) included increased relative liver weight (17% in the high-dose group only); hypertrophy of the centrilobular zone of the liver (high-dose group only); increased nuclear volume of hepatocytes and proliferation of smooth endoplasmic reticulum (only the high-dose and control groups were evaluated in this report); increases in the concentrations of cytochrome P-450 and cytochrome b5 (i.e., at the mid- and high-dose groups); increases in the enzymatic activities of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH)-cytochrome c-reductase, alanine p-hydroxylase, succinate dehydrogenase and aminopyrine N-demethylase (mid- and high-dose groups); and decreased hexobarbital sleeping time (mid- and high-dose groups). In general, maximal effects were achieved by 6 weeks of exposure and control levels returned after a 4-week solvent-free period following the 6-month exposure.

Continuous inhalation exposure of CFY rats to 0, 350, 460, or 1150 ppm (i.e., 0, 1500, 2000, or 5000 mg/m^3) mixed xylenes (comprising 10% *ortho*-xylene, 50% *meta*-xylene, 20% *para*-xylene, and 20% ethylbenzene) for 72 hours or following repeated inhalation exposure of male mice, rats, or rabbits to 0 or 575 ppm (2500 mg/m^3) for 8 hours per day for 6 weeks resulted in effects similar to those reported for the repeated exposure study in male rats for 6 months that was described above (U.S. EPA 2003).

Subchronic or inhalational exposure of rats, guinea pigs, dogs, and monkeys to mixed xylenes and *ortho*-xylene alone were observed to provoke adverse renal effects (ATSDR 2007 and 2016). At xylene concentrations of 50 to 2000 ppm, it was shown that inhalational exposure provoked increased renal enzyme activity, increased renal cytochrome P-450 content, and increased kidney-to-body weight ratios (i.e., in *ortho*-xylene-exposed rats). However, a histopathological study of rats, guinea pigs, dogs, and monkeys failed to demonstrate any evidence of renal lesions after inhalation of 810 ppm mixed xylenes or 78-ppm *ortho*-xylene

following a subchronic exposure (i.e., representing an intermediate period of 13 weeks and 90 to 127 days, respectively). Absolute or relative kidney weights were not affected in male rats that had been intermittently exposed to *meta*-xylene at concentrations as high as 100 ppm for 13 weeks (ATSDR 2016).

2.4.1.7 Mammalian Inhalation Toxicity—Chronic

Inhalation exposure of rats to 300-ppm technical xylene (*para*-xylene 19.5%, *meta*-xylene 43.0%, *ortho*-xylene 19.2%, and ethylbenzene 18.3%) for 18 weeks initially caused xylene to accumulate in the perirenal fat, but after the first 2 weeks of exposure, concentrations declined (Elovaara et al. 1980). Adult male rat exposure to 300-ppm xylene containing 80% of *meta*-xylene and 12% *para*-xylene for 6 hours a day 5 days a week for 2 weeks increased xylene accumulation in the perirenal fat as compared to 1 week of exposure (Savolainen et al. 1978).

In a related study, exposure in rats to 300 ppm, 5 days a week, for 18 weeks did not produce liver damage as shown through histological examination (Elovaara et al. 1980). Inhalation exposure to 300-ppm technical xylene (5% *meta*-xylene with the remaining 15% comprised of *ortho*- and *para*-xylene) for 6 hours a day for 5 days a week for up to 18 weeks had no effect on body weight gain or water consumption. After 9 weeks of this exposure, xylene-treated rats showed reductions in preening frequency and duration (Savolainen et al. 1979).

2.4.1.8 Mammalian Inhalation Toxicity—Developmental

Pregnant rats exposed to 1000 mg/m^3 (230 ppm) during days 9–14 of pregnancy exhibited no maternal toxicity. No adverse effects observed for mean litter size, mean fetal weight, or mean placental weight. No adverse effect on the number live fetuses at day 21 (14.3 per female in treated, 13.4 in controls), number of fetuses resorbed (0.75 per female in treated, 0.6 in controls), or number of dead (0.05 per female in treated, 0 in controls). No increase was observed in the number of fetuses with external malformation or internal malformations. An increase existed in the numbers of fetuses with skeletal anomalies: 8/146 fetuses from treated females had fused sternebrae; whereas, the number was 2/179 in controls; 9/146 had extra ribs in fetuses from treated females, with 0/179 in controls. No effect was observed on skeletal malformations. No teratogenic effect of these solvents was found in continuous exposure to 1000 mg/m^3 (230 ppm) xylene from day 9 to 14 of pregnancy; however, embryotoxicity was evident in the form of skeletal anomalies (Hudák and Ungváry 1978).

Pregnant rats exposed via inhalation to 10, 50, or 500 mg/m^3 (2.3, 11, and 110 ppm) 6 hours a day 5 days a week throughout pregnancy. Of these, females exposed to 50 or 500 mg/m^3 exhibit developmental and teratogenic effects. Exposure to 10 mg/m^3 did not exhibit similar effects. Exposure to 50 and 500 mg/m^3 caused increased post-implantation losses, significantly decreased fetal weight; increased incidence of hemorrhages, many in the cervical region. Teratogenic effects at 500 mg/m^3 included hydrocephalus, microphthalmia, intracerebral hematomas, dilations of the aorta and auricles of the heart, and hemorrhages of the liver. Exposure to 50 and 500 mg/m^3 lead to increased incidences of abnormal ossification of the sternum, impaired formation of the skull, and impaired ossification of the parietal and interparietal bones. Offspring development was impaired at 50 and 500 mg/m^3 as indicated by

delayed physical maturation and functional inferiority of various organs. Mean weight in fetuses was decreased at days 7 and 21. Metabolism in liver, brain, myocardium, and lungs was disturbed at 50 and 500 mg/m^3 (Mirkova et al. 1983).

When time-mated Wistar rats were exposed to 200 ppm via inhalation for 6 hours per day on days 4–20 of gestation, no effects of exposure were observed on viability of the progeny assessed as number of fetuses or pups per litter, neonatal death and survival until day 28, or on the frequency of malformations. Basically, mixed xylene was not teratogenic via inhalation at 200 ppm when exposed throughout pregnancy (Hass and Jakobsen 1993). In another study, exposing pregnant females to 500 ppm from days 7–20 of pregnancy (Hass et al. 1995), only slight impairment of development of motor skills or air righting reflexes were noted. Again, no signs of maternal, reproductive or development toxicity were reported.

A teratogenicity test using a xylene mixture containing: sulfur at 0.4 ppm; toluene at 0.12 wt.%; ethylbenzene at 36.08%; *para*-xylene at 0.31%; *meta*-xylene at 52.07%; *ortho*-xylene at 1.40%; and C9 saturates at 0.02% ultimately found minimal teratogenic effects following exposure. Female rats were exposed via inhalation to xylene at concentrations of 0, 100, or 400 ppm. No maternal death or other signs of toxicity were observed. Very few abnormalities of offspring were noted and these were not considered compound related. One subcutaneous hematoma in a single control offspring was noted. A single offspring from a female exposed to 100 ppm of the xylene mixture died and demonstrated the appearance of exencephaly, which is a cephalic disorder in which the fetal brain is located external to the skull. The offspring in one litter from a female exposed to 400 ppm were undersized. A single offspring from a female exposed to 400 ppm had no eyes. There was an increase in skeletal abnormalities in offspring from females exposed to 400 ppm, mostly related to retarded bone ossification. The authors conclude exposure of the test material by inhalation to female rats at doses of 0, 100, and 400 ppm produced no effect on the pregnant dams. There was no evidence of variation in fetal sex ratio, embryo toxicity or inhibition of fetal growth and development, or teratogenic potential resulting from exposure of the dams to these concentrations of xylene (Litton Bionetics Inc.® 1978b).

Mice, rats, and rabbits were exposed to xylene via inhalation during pregnancy. The mice were exposed for 3 periods of 4 hours each day on days 6–15 of pregnancy at either 500 or 1000 mg/m^3 (110 or 230 ppm) and exhibited no differences in number of live fetuses, dead or resorbed fetuses, or animals with minor anomalies or extra ribs. Female mice exposed to 230 ppm exhibited increased number of fetuses with retarded weights and increased numbers with retarded skeletal growth. Rabbits were exposed to 500 or 1000 mg/m^3 (110 or 230 ppm) xylene for 24 hours a day on GDs 7 through 20, following which they exhibited no effect on maternal body weight. Exposure to 230 increased maternal relative liver weights. Of the 10 female rabbits expose to 1000 mg/m^3, 3 died, 6 had abortions, and 1 resorbed all fetuses. No viable fetuses were found in female rabbits that were exposed to 230-ppm xylene. However, exposure to 110-ppm xylene provoked reduced body weight in adult females without showing evidence of increased teratogenic or reproductive effects. Rats were exposed to 250, 1900, or 3400 mg/m^3 (57, 430, or 770 ppm) xylene for 24 hours on GDs 7 through 15. Female rats that were exposed to 770 ppm had fewer viable fetuses, significantly more non-viable or resorbed fetuses, an increased number of fetuses with reduced body weight, and an increased number of fetuses

with minor anomalies or extra ribs. All dose groups of rats presented with increased numbers of rats showing retarded skeletal growth (Ungváry and Tátrai 1985).

The embryotoxic effects of xylene were studied by others following exposure of rats to 1000 mg/m^3 of air during days 9 through 14 of pregnancy. Following exposure, no teratogenic outcomes were observed, although minor skeletal anomalies occurred (Shepherd 1986). Similarly, others have explored embryotoxicity in a rat model following exposure to xylene at 50 or 500 mg/m^3 (Mirkova et al*.* 1983). This group showed that exposure to xylene was indeed embryotoxic and displayed teratogenic effects following treatment. The brain, liver, lungs, and heart were affected. The number of post-implantation losses increased by 9.7% and 168% in the 50 and 500 mg/m^3 treated xylene groups, respectively. In addition, the incidence of fetal skeletal abnormalities increased by 62% and 177% in the 50 and 500 mg/m^3 treated xylene groups, respectively (Mirkova et al. 1983).

2.4.1.9 Mammalian Inhalation Toxicity—Reproduction

In a reproductive toxicity test, adult rats were exposed 6 hours a day via inhalation in whole body exposure chambers to 60, 250, and 500 ppm for a 130-day pre-mating period, 20-days mating period, during gestation, and then pups were exposed for days 5–20 of lactation. No effects were observed on adults or offspring exposed to 60 ppm. No treatment-related mortality occurred at any exposure level. Numbers of females mating and becoming pregnant were reduced in rats exposed to 250 ppm, but the effect was not consistent in those at 500 ppm.

In pairs where only the female was exposed to 500 ppm, reproductive indices were reduced; however, when only the male or both male and female of a pair were exposed to 500 ppm, the same effects were not observed. No other adverse effects occurred at 250 ppm. Zero filial generation (F_0) females exhibited reduced kidney weights at 500 ppm. Fetal weights were lower when only the female of the pair was exposed to 500 ppm. Exposure to 500 ppm increased the incidence of ossification variations. First filial generation (F_1) ovary weights were reduced at day 21, but no difference from controls was apparent at day 49. No other adverse effects were observed (Bio/Dynamics 1982).

In a comprehensive one generational reproductive study conducted in CD rats, one-half of the group I generation F_0 pregnant dams (20 females; control group) and group IV generation F_0 pregnant dams (that comprised 12 females that exposed by inhalation to 500 ppm mixed xylenes for 6 hours per day, and 5 days per week, during a premating period and during gestation) were sacrificed on GD 21 to evaluate any evidence of developmental toxicity (U.S. EPA 2003). Gross necropsy was conducted on each animal.

Maternal exposure to 500-ppm mixed xylenes did not adversely affect maternal body weights, food consumption, or utilization and did not affect the results of post-mortem examination. Terminal body weights (that were corrected for gravid uterine weights) for exposed females were statistically, significantly increased when compared with those of controls. However, the increases were thought not to be biologically significant (106% of controls). Although absolute kidney weights were statistically increased in-group IV females (110% of controls), kidney weights relative to body weights were comparable to those found in controls.

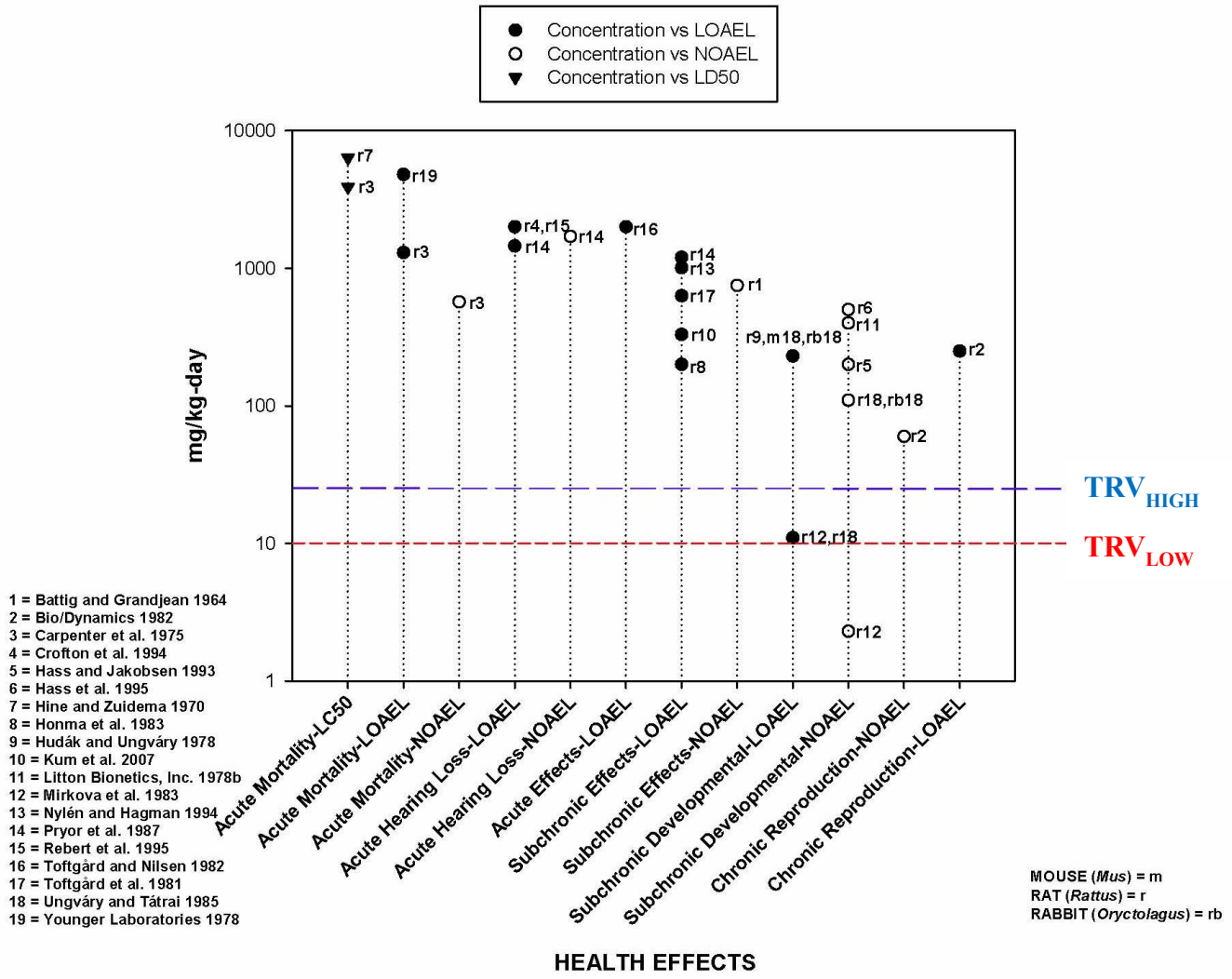


Figure 2. Mixed Xylenes: Inhalation Effects to Mammals

Consequently, the increase in absolute kidney weights in the exposed females was attributed to the higher body weights. No statistically significant differences were noted between treated (i.e., group IV) and control groups for the mean number of corpora lutea, implantations, live fetuses, mean percentage of viable fetuses/implants, or fetal sex ratios. Although the exposed group had an increased mean number of resorption sites (1.6 vs. 1.2 for controls) and mean percentage of resorptions to implants (16.2% vs. 9.9% for controls), the increases were not statistically significant. None of the dams demonstrated whole litter resorption. No definitive treatment-related external, visceral, or skeletal malformations or variations were observed. Mean fetal body weights on GD 21 were marginally but statistically significantly decreased in exposed female fetuses (i.e., 93% of controls); however, male fetal weights were comparable to those found in control animals. The observed apparent decreases in mean fetal body weights were thought not to be biologically significant (U.S. EPA 2003).

2.4.1.10 Mammalian Inhalation Toxicity—Neurotoxicity

Inhalational exposure of pregnant female rats to 500 ppm, for 6 hours a day on days 7–20 caused lasting neurobehavioral developmental effects. Twelve-week-old young female rats that had been exposed in-utero exhibited delayed response times in a Morris water maze test. The Morris water maze task requires rats to spatially navigate, using cues to locate a small platform under the surface of the water in a large pool. Depending on the conditions of the test, the exposed offspring showed delayed response times in some trials. The inability to find the hidden platform was not due to poor swimming but due to problem solving abilities (Hass et al. 1997).

Inhalational exposure to mixed xylenes at 2400 or 7000 ppm for 30 minutes increased both the rapid (< 2 second) inter-response times and activity pauses (of 22 seconds or greater) in mice trained to press a lever to receive sweetened milk (Moser et al. 1985). Ghosh et al. (1987) found that concentrations as low as 114 ppm could alter the behavior of rats. In this study, the same rats pressed a lever to receive sucrose less frequently during exposure periods than during control sessions.

Mongolian gerbils (*Meriones ungiculatus*) were exposed to 160 and 320 ppm of xylene comprised of 18% *ortho*-xylene, 70% *meta*-xylene and 12% *para*-xylene, analytical grade (96% pure), containing less than 3% ethylbenzene and less than 0.1% toluene. Exposures were continuous for 3 months. Brain damage was evident as seen by increased expression of two-astroglial cell marker proteins glial fibrillary acid protein (GFA) and S-100 following exposure to 350 ppm and decreased DNA concentrations in various portions of the brain, including the cerebral hemispheres, also following exposure to 350 ppm (Rosengren et al. 1986).

In experimental studies conducted in a variety of animals, comprehensive evidence indicated the neurotoxicity of mixed xylenes and its component isomers following inhalation exposure. Signs of neurotoxicity were observed in rats, mice, dogs, cats, and gerbils following acute and subchronic inhalational exposure to various xylene isomers. Neurotoxicological end-points included observation of central nervous system (CNS) depression, prostration, incoordination, tremors, and muscular spasms, labored breathing, hyper-reactivity to stimuli, and decreased acetylcholine levels in the midbrain and norepinephrine in the hypothalamus, among several other neurocognitive and behavioral effects (ATSDR 2007). These observations suggested an

effect on motor control, sleep, and memory maintenance. A comparative study determined that the minimal alveolar concentrations needed to induce anesthesia in rats were similar for all three isomers (0.00118, 0.00139, and 0.00151 atm [atmosphere], respectively, for *ortho*-, *meta*-, and *para*-xylene); however, only *para*-xylene also induced excitation (i.e., as exemplified by strong tremors; ATSDR 2007).

Corroborating the above neurotoxicological effects was a recent article that comparatively determined the structure-activity effects of a number of similar hydrocarbon solvents and subsequent CNS manifestations (Armenta-Resendiz et al. 2019). Male Wistar rats were assessed for the effects of hydrocarbon solvents that were chemically related to toluene on anxiety-like behavior, passive-avoidance learning, nociception, motor coordination, and social interaction.

After a 30-minute inhalational exposure to one of cyclohexane, benzene, toluene or m-xylene (at doses of 2000 to 8000 ppm), independent groups of adolescent male rats were assessed for their abilities to show burying behavioral tasks, a step through avoidance learning task, the hot plate test, the shock threshold test, and their social interaction behaviors or rotarod tests as compared rats that were exposed only to air. Rats exposed to benzene, toluene, and m-xylene produced anxiolytic-like actions, impaired learning, caused antinociception, and decreased social interaction in a concentration-dependent manner. Locomotor coordination was impaired only with 8,000-ppm m-xylene and 8,000-ppm toluene. Unsurprisingly, the aromatic ring was found to be critical for eliciting a variety of behavioral effects (Armenta-Resendiz et al. 2019).

2.4.2 Dermal Toxicity

The acute dermal LD_{50} in rabbits is reported to be 1700 mg/kg for mixed xylenes (U.S. EPA 2005b), but a critical review of the document did not identify the source of the reported value. It appeared that the dermal toxicity value was derived for an oral toxicity value. Younger Laboratories (1978) estimated the acute dermal LD_{50} to be >3150 mg/kg, but very few animals were tested. No mortality occurred at 2000 mg/kg (0/2) or 3160 mg/kg (0/1); 1/1 at 5010 mg/kg; 1/2 7940 mg/kg. Clinical signs included weight loss, increasing weakness, collapse, and death. Necropsy findings included hemorrhagic areas of lungs, liver and kidney discoloration, enlarged gall bladder, and gastrointestinal inflammation (Younger Laboratories 1978).

Dermal LD_{50} was estimated at greater than 20,000 mg/kg in albino rabbits; however, only 4 animals were tested per dose group (5000, 10,000 and 20,000 mg/kg). Rabbits dosed at 20,000 mg/kg exhibited pain, depression, hyperpnea, severe edema and erythema, and cyanotic spots with areas of eschar. Necropsy in dead rabbits showed severe hemorrhage and congestion of lungs. In survivors, the skin was not healed at 14 days with no other gross pathology (Procter and Gamble Company 1978).

Female hairless albino mice were treated topically for 5 days per week for either 8 weeks or 6 months with 100 microliter (µL) of 50% xylene in mineral oil. The effects after 8 weeks were similar to those following 6 months of exposure, only less severe. After 6 months, the mice exhibited a great increase in intensely granular, large mast cells, associated with an infiltrate of

lymphocytes. The epidermis thickened with hypertrophic keratinocytes, among which lymphocytes had intruded (exocytosis) (Kligman and Kligman 1998).

Only 0.12% of the applied dose of xylene was absorbed into the skin of weanling pigs when xylene was applied with J-8 jet fuel as the carrier. The jet fuel could have affected the rate at which the xylene was absorbed, so that absolute absorbed amount is not what is important. The study results indicate that xylene will penetrate mammalian skin. Exposure to xylene in J-8 jet fuel also caused temporary skin irritation, but it is not clear whether the irritation was due to the presence of the jet fuel or the xylene (Singh et al. 2003).

Repeated applications of 0.25 milliliter (mL) on areas where the skin had been clipped caused mice to exhibit edema and dry scaliness. The mice became very irritable after the application of xylene and engaged in a considerable amount of backbiting. No ulceration appeared to result from the xylene, but xylene appeared to have some toxicity for the mice, eight (8/10) of which died in the 2 weeks after the application (Pound and Withers 1963).

2.4.3 Mammalian Toxicity—Other

2.4.3.1 Mammalian Toxicity—Other: Teratogenicity

In separate *in vitro* studies, Brown-Woodman et al. (1991, 1994) cultured rat embryos in rat serum containing xylene. Brown-Woodman et al. (1991) determined that rat embryos exposed to as little as 0.09-µL xylene/mL of serum can cause embryotoxicity. Reduced crown-rump measurements occurred following 48 hours of exposure. Over the test period, the serum concentration diminished to 0.05-µL xylene/mL serum. They did not identify any teratogenic effects when embryos were cultured in serum containing xylene at a concentration of up to 0.57 µL/mL (0.49 mg/mL). Over the 48 hours of exposure, this initial concentration diminished to 0.28 µL xylene/mL serum (0.24 mg/mL). A reduction in the number of somites was noted at 0.24 µL xylene/mL serum (0.21 mg/mL; reducing to 0.15 µL/mL or 0.13 mg/mL).

In a second study with similar concentrations of xylene in a culture media of rat serum, Brown-Woodman et al. (1994) found that initial concentrations of 0.5-µL xylene/mL serum (confirmed as 1.89 micromole per heart rate per milligram (µmol/mL) [0.2 mg/mL] reducing to 0.67 µmol/mL (0.07 mg/mL) after 40 hours) disrupted development of yolk-sac circulation, reduced crown-rump length, reduced the number of somites, and reduced the protein per embryo. A dose of 0.5-µL xylene/mL serum (0.2 mg/mL) was considered the LOAEL. Initial xylene concentrations of 0.2-µL xylene/mL serum (confirmed as 1.08 µmol/mL (0.11 mg/mL) reducing to 0.60 µmol/mL [0.06 mg/mL] after 40 hours) did not cause adverse effects and was considered the NOAEL.

2.4.3.2 Mammalian Toxicity—Other: Mutagenicity

When exposed to 300 ppm, via inhalation, for 6 hours a day, 5 days a week, for total exposure periods of 9, 14, or 18 weeks, no indication of chromosomal aberrations was evident in bone marrow (Donner et al. 1980). Xylene was negative for chromosome aberrations (CA) and sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) (Zeiger et al. 1990) cells at test concentrations up to 100 µg/mL for CA and up to 50 µg/mL for SCE (Anderson et al. 1990) or up to 1520-µg/mL xylene for SCE (Gerner-Smidt and Friedrich 1978). Results for both CA and

SCE were negative with and without the addition of an exogenous metabolic activation (S9) mixture. Concentrations greater than 100 µg/mL or 50 µg/mL were toxic for CA and SCE, respectively (Anderson et al. 1990).

When mixed xylenes was tested for induction of mutations in *Salmonella* and induction of mutations in mouse lymphoma L5178Y cells, the only positive results were for mouse lymphoma cells in the presence of S9 supernatant. All tests in the absence of S9 were negative (Lebowitz et al. 1979, Zeiger et al. 1990). Concentrations of 75 or 105 µg/mL caused positive response in the L5178Y mouse lymphoma cell mutation assay (Myhr et al. 1990).

Mixed xylenes showed moderate toxicity to mouse BALB/c-3T3 cells with an LD_{50} of 3.20 millimolar (mM) (Matthews et al. 1993). Mixed xylenes were categorized as inactive for transformation of BALB/c-3T3 cells (Matthews et al. 1993). No increase in transformation occurred in mouse T3T cells in the presence of herpes simplex type 2 virus at concentrations of xylene up to 0.5 µL/mL (equivalent to a dose of 0.43 mg/mL) (Johnson 1981).

Lebowitz et al. (1979) tested mixed xylenes *in vivo* with the rat bone marrow chromosome aberration assay. When used *in vivo* and administered intraperitoneally, xylene failed to show evidence of clastogenic activity in bone marrow.

Litton Bionetics, Inc. (1978a) conducted three tests of mutagenicity with xylene using a mixture containing: sulfur--0.4 ppm; toluene--0.12%; ethylbenzene--36.08%; *para*-xylene--0.31%; *meta*-xylene--52.07%; *ortho*-xylene--11.40%; and C9 saturates--0.02%. Xylene was not mutagenic for the *Salmonella* mutant series in the plate or suspension assays conducted at concentrations from 0.000463% to 0.0037%. Xylene was not mutagenic when tested for forward mutation in TK+/-L5l78Y mouse lymphoma cells. The dose range was adequate to demonstrate cytotoxicity. Concentrations for non-activation tests ranged from 0.00640 µL/ml to 0.1 µL/mL (0.0055 to 0.086 mg/mL). Concentrations for activation tests ranged from 0.02500 µL/ml to 0.15 µL/mL (0.022 to 0.13 mg/mL). Xylene did not produce significant increases in chromosome aberrations in rat bone marrow cells at concentrations of 0.044, 0.147 and 0.441 mL/kg (i.e., equivalent to 37.8, 126, and 379 mg/kg).

2.4.3.3 Mammalian Toxicity – Other: Neurotoxicity

Pathogen-free Sprague-Dawley rats were exposed to 2000-ppm mixed xylene (2, 64.5, 10, 23, and 0.5% of *ortho*-xylene, *meta*-xylene, *para*-xylene, ethylbenzene, and toluene, respectively) for 6 hours on 3 consecutive days. Following exposure, animals produced increased dopamine levels within the center of the nucleus caudatus, which is located within the marginal zone of the nucleus caudatus, and within the dopamine-cholecystokinin-8-like immunoreactive nerve terminals of the posterior nucleus accumbens and of the medial-posterior tuberculum olfactorium. Xylene also produced a significant increase in noradrenaline levels in the parvocellular part of the paraventricular hypothalamic nucleus and within the subependymal layer of the median eminence and an increase in the dopamine fluorescence in the lateral palisade zone. Xylene can change dopamine levels and turnover in various striatal and limbic dopamine terminal systems indicating that xylene, via disturbing dopamine neurotransmission in striatum and limbic areas, can produce motor disturbances and motivational deficits. However,

this exposure to 2000-ppm mixed xylenes produced no-observable changes in behavior (Andersson et al. 1981).

When 68 male albino rats (weight of approximately 100–120 g) of 16 rats per group were treated subcutaneously with xylene at doses of up to 0.10 ml/100 g-body weight (860 mg/kg), there was no effect on the ability of rats to run a maze that had already been learned even after 28 days of dosing. Doses were administered immediately following running the maze each day. However, a dose of 0.05 mL/100 g-body weight (430 mg/kg) administered subcutaneously was able to inhibit a rat's ability to learn the maze. Running times for treated rats did not approach those of the control rats until 40 days after treatment started (Dési et al. 1967).

2.5 Summary of Avian Toxicology

Fertile mallard eggs dipped in either 1% or 10% xylene suspensions on days 3 or 8 of incubation had insignificant effects on embryonic survival, embryonic weight and length or percent survivors that were abnormal through day 18 of incubation (Hoffman and Eastin 1981).

A dietary toxicity test of 5 days, started when Japanese quail (*Coturnix japonica*) chicks were 14 days old did not elicit any overt signs of toxicity at 5000 ppm (Hill and Camardese 1986). Breeding Japanese quail were fed diets containing xylene for 2 months providing a daily dose of 3 mg/kg/day. Body weights of the males receiving the treated diets were reduced, but female body weights showed no difference from controls. The weights of the testes and ovaries increased in the quail receiving the treated diets. The treated diet did not reduce fertility of eggs laid but did cause an increase in embryonic mortality and reduced the percentage of eggs hatching. Weights of the ovaries and testes increased in 14-day-old embryos (David 1982).

Japanese quail were treated dermally with an unstated/unknown quantity of mixed xylenes. Quail treated with xylene showed dramatically reduced production of anti-sheep red blood cell antibodies compared to controls indicating a transient suppression of the humoral immunity in quail (Singh et al. 1994).

2.6 Summary of Amphibian Toxicology

Kononen and Gorski (1997) performed frog embryo teratogenesis assay-Xenopus (FETAX) testing with mixed xylenes. The use of static renewal exposures for the frog embryos with a highly volatile chemical creates doubts regarding the validity of the reported LC_{50}, $EC_{50,}$ or lowest-observed effect concentration (LOEC) values. However, there seems little doubt that exposure to mixed xylenes caused skeletal abnormalities; cardiac, gut, optic, and facial edema; microcephaly; microphthalmia; abnormal pigmentation; abdominal blistering; and abnormal gut coiling. In general, the frequency of these malformations increased with increasing mixed xylenes concentrations. Test concentrations were not definitively stated, but were at least up to 118 mg/L.

Black et al. (1982) conducted toxicity testing with leopard frog eggs and larvae under flow-through conditions. Exposure was initiated within 30 minutes of fertilization. Hatchability was reduced at 1.43 and 3.16 mg/L and almost eliminated (9%) at 33.6 mg/L. No larvae survived at

33.6 mg/L. At 3.16 mg/L, survival was 80% at hatching and 74% 4 days after hatching. All hatched larvae at 33.6 mg/L were malformed, but exposures less than that did not appear to increase teratogenic effects. The LC_{50} was estimated to be 3.53 mg/L.

In another study (Gao et al. 2016), healthy tadpoles (*Xenopus laevis*) were exposed to *para*-Xylene across four doses (1, 2, 2.5 and 3 mM) and a control buffer solution. The lethal toxicity of *para*-xylene was studied, from which the concentration that caused 50% mortality (LC_{50}) in the test population at 72-hour post-exposure was estimated as 1.94 mM. In studies again employing the FETAX assay, apoptosis mode of cell death assays, and visual avoidance tests, exposure to *para*-Xylene induced a dose-dependent increase in mortality, was neurotoxic to the central nervous system, and increased the rate of abnormal development (Gao et al. 2016).

Although toxicological studies of mixed xylenes on amphibians remain poorly studied, one relatively recent study (Sutuyeva et al. 2019), investigated the mortality of embryos and the developmental effects in the marsh frog (*Rana ridibunda*) following aquatic exposure to the water-soluble fraction of crude oil as compared to o-xylene. Developmental (subchronic) effects were explored by exposing *R. ridibunda* eggs at the glutenin subunits (GS) 8–11 stage of development to o-xylene at 0.05, 0.5 or 1.5 mg/L and a control exposure (ethanol at 0.005% as carrier vehicle). The group also conducted chronic exposures to o-xylene at 0.05, 0.5, or 1.5 mg/L on GS 26 stage tadpoles (Sutuyeva et al. 2019).

On exposure to o-xylene, developmental delay and axial curvature were noted in the tadpoles. At o-xylene exposures of 0.5 and 1.5 mg/L, the incidence of gut uncoiling and edema was the highest (39% and 42% respectively) as compared the control (Sutuyeva et al. 2019). In chronic exposure studies, the mortality rate never exceeded 7% in control exposures to o-xylene. However, in tadpoles chronically exposed to 0.5 or 1.5 mg/L, o-xylene exhibited a significantly depressed body weight (28 and 48 percent respectively). Exposure to o-xylene also provoked significant developmental delays in tadpoles, particularly at the 0.5 and 1.5 mg/L exposure levels. For example, 17% and 33% respectively decreased both snout-vent length and total body length when exposed to 0.5 or 1.5 mg/L-o-xylene (Sutuyeva et al. 2019). Interestingly, observed effects demonstrated that the embryotoxic and teratogenic effects attributed to o-xylene exposure might have been mediated via oxidative stress mechanisms.

2.7 Summary of Reptilian Toxicology

No toxicological data for the effects of mixed xylenes on reptiles was located.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Acute toxicity studies for ingestion of mixed xylenes indicate that LD_{50} values in rats range from 3523 to 8700 mg/kg and in mice from 5251 to 5627 mg/kg (Table 2). Clinical signs such as weight loss, increasing weakness, collapse and death as well as gross pathology findings of lung and liver hyperemia, discoloration of kidneys and spleen, and gastrointestinal inflammation are affected as doses as low as 3160 mg/kg (Younger Laboratories 1978).

Subchronic oral gavage studies with rats and mice ranged in duration from 10 days of dosing to 14 days and up to 13 weeks (Table 3). Mortality was reported to be as low as 2000 mg/kg-day in both mice and rats (NTP 1986). Decreased body weights occurred in rats at 125 mg/kg-day for females and 250 mg/kg-day for males (NTP 1986). Chronic 2-year exposure studies in rats and mice caused an increased mortality at a dose of 500 mg/kg-day in male rats, and 1000 mg/kg-day in female mice with no increased mortality or other clinical signs observed at 250 mg/kg-day in rats and 500 mg/kg-day in mice (NTP 1986). No evidence of carcinogenicity was found for mixed xylenes when tested in both sexes of rats and mice in the 2-year chronic study. Only two oral dose levels (i.e., at 250 and 500 mg/kg) in rats and mice are available, and there was no provision of quality dose response data to consider BMD analysis from these exposures. The combined NOAEL for these studies was 250 mg/kg-day, which was derived from the subchronic study

The above discussion shows that the subchronic toxicity outcomes are comparatively more toxic than the chronic toxicity data. Thus, the Technical Guide 254 (USACHPPM 2000) provides uncertainty factors (UF) of 20 for the NOAEL TRV and 4 for the LOAEL TRV when the TRVs are based on a subchronic LOAEL. Use of these UFs and the LOAEL of 125 mg/kg-day results in a NOAEL TRV of 6.25 mg/kg-day and a LOAEL TRV of 31.25 mg/kg-day (NTP 1986).

Table 6. Selected Ingestion TRVs for Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	6.25 mg/kg-day	Moderate
TRV_{High}	31.25 mg/kg-day	Moderate

Legend:
NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Source: NTP 1986

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Acute toxicity studies of mixed xylene inhalation indicated LC_{50} values in rats ranging from 3,900 to 10,950 ppm (Table 4). Clinical signs including incoordination occurred at exposures as low as 1300 ppm (Carpenter et al. 1975) and hearing loss at exposures as low as 1450 ppm (Pryor et al. 1987). Only one study reported a NOAEL—at 570 ppm (Carpenter et al. 1975).

Subchronic inhalation studies with rats range in duration from 21 days of dosing to 14 days and up to 12 weeks (Table 5). No treatment-related mortality was reported in any of the subchronic inhalation studies. Significant increases in GABA [γ-aminobutyric acid] in brain tissue and significant decreases in dopamine in the striatum occurred at 200 ppm (Toftgård et al. 1981), and renal toxicity was noted at 330 ppm (Kum et al. 2007).

Chronic exposures in rats for 18 weeks at a dose of 300 ppm did not produce any adverse effects (Savolainen et al. 1979, Elovaara et al. 1980). Since no effects were observed in the chronic studies, and adverse effects were noted at the same or lower exposures in subchronic tests, the subchronic results were used to develop the TRVs. The TRV Protocol (USACHPPM 2000) provides UFs of 20 for the NOAEL TRV and 4 for the LOAEL TRV when the TRVs are based on a subchronic LOAEL. Use of these UFs and the LOAEL of 200 ppm results in a NOAEL TRV of 10 ppm and a LOAEL TRV of 50 ppm.

Table 7. Selected Inhalation TRVs for Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	10 ppm	Moderate
TRV_{High}	50 ppm	Moderate

Legend:
NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Source: Toftgard et al. 1981

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Acute dermal LD_{50} values in rabbits are reported to range from 1700 mg/kg (U.S. EPA 2005b), > 22150 mg/kg (Younger Laboratories 1978), and > 20,000 mg/kg (Procter and Gamble Company 1978). None of these values were judged reliable. Therefore, these studies do not provide sufficient information to determine a TRV for dermal exposure at this time.

3.2 Toxicity Reference Value for Birds

A single acute dietary study failed to identify any adverse effects following exposure to mixed xylenes at 5000 ppm in the diet (Hill and Camardese 1986). A single dietary study that had reported on dosing adult quail with the equivalent of 3 mg/kg-day produced reproductive impacts such as increased embryonic mortality (David 1982), but only the single dietary

concentration was tested. Additional avian toxicity studies report effects following application to the eggshell or injection into eggs. No studies with described impacts reported a range of doses suitable for dose-response modeling. Therefore, insufficient data are available to determine a TRV for birds at this time.

3.3 Toxicity Reference Values for Amphibians

The only amphibian toxicity studies available (Black et al. 1982, Kononen and Gorski 1997; Gao et al. 2016 and Sutuyeva et al. 2019) tested for effects of embryos to xylene in water. Thus, no suitable studies are available to determine a terrestrial wildlife TRV. However, in a limited dataset (Gao et al. 2016), a tentative 72-hr LC_{50} of 1.94 mM was determined.

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

3.5 Important Research Needs

The lack of data on the toxicity of mixed xylenes to wildlife species weakens the development of a TRV. Hence, additional toxicological studies of the compound and its derivatives are recommended. An unusually large literature base exists for inhalation in mammals, but for no other taxonomic group. Additional laboratory mammal testing is not necessary for inhalation toxicity, but is still lacking for wild species and for birds, reptiles and amphibians. Adequate dermal toxicity data are lacking for all groups. The toxicity literature is scant for birds and amphibians, and completely lacking for reptiles. Thus, studies that focus on both acute and chronic toxicity studies on wild mammals as well as non-mammalian wildlife such as birds, reptiles and amphibians are particularly warranted.

APPENDIX A

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APPENDIX B

LITERATURE REVIEW

A very broad search on September 9, 2010 using DTIC's Multisearch function used the single search term, xylene. This search identified 2319 documents.

Additional searches on September 9, 2010 using DTIC's Multisearch function used the terms:

xylene + quail*. This search identified 15 documents.
xylene + mallard*. This search identified 21 documents.
xylene + bird*. This search identified 179 documents.
xylene + avian. This search identified 29 documents.
xylene + mouse. This search identified 120 documents.
xylene + mice. This search identified 120 documents.
xylene + rat. This search identified 124 documents.
xylene + mammal*. This search identified 135 documents.
xylene + ecotox*. This search identified 46 documents.
xylene + toxic*. This search identified 1128 documents.
xylene + amphib*. This search identified 126 documents.
xylene + frog. This search identified 35 documents.
xylene + *Xenopus*. This search identified 2 documents.
xylene + reptil*. This search identified 55 documents.

The same broad search on September 21, 2012 using DTiC's Multisearch function used the single search term, xylene with a limit of published articles from 2010 to 2012. This search identified 1702 documents.

Additional searches on September 21, 2012 using DTiC's Multisearch function used the terms:

xylene + quail*. This search identified no new documents.
xylene + mallard*. This search identified no new documents.
xylene + bird*. This search identified no new documents.
xylene + avian. This search identified no new documents.
xylene + mouse. This search identified one new documents.
xylene + mice. This search identified five new documents.
xylene + rat. This search identified seven new documents.
xylene + mammal*. This search identified no new documents.
xylene + ecotox*. This search identified no new documents.
xylene + toxic*. This search identified no new documents.
xylene + amphib*. This search identified no new documents.
xylene + frog. This search identified no new documents.
xylene + *Xenopus*. This search identified no new documents.
xylene + reptil*. This search identified no new documents.

On September 9, 2010, a search of the U.S. EPA's online Ecotox database used the CAS No. 1330-20-7. One reference for amphibians, and no reptile or bird references were identified. Eight mammalian references were found.

On September 19, 2012, a search of the U.S. EPA's online Ecotox database used the CAS No. 1330-20-7 to identify any new articles since the search performed in 2010. One reference for amphibians and one bird reference were identified. Eight mammalian references were found. No references were found for reptiles. No new references were identified from later than 2010.

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on September 9 and 10, 2010 used the CAS No. 1330-20-7 as the search term. A total of 7920 articles were identified. This search was refined with:

1330-20-7 and ecotox* resulted in 46 hits
1330-20-7 and reptil* resulted in 1 hit
1330-20-7 and amphib* resulted in 9 hits
1330-20-7 and *Xenopus* resulted in 10 hits
1330-20-7 and frog resulted in 7 hits
1330-20-7 and avian resulted in 31 hits
1330-20-7 and mallard resulted in 3 hits
1330-20-7 and quail resulted in 10 hits
1330-20-7 and bird* resulted in 29 hits
1330-20-7 and wild* resulted in 63 hits
1330-20-7 and mammal* resulted in 244 hits

A second search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on September 19, 2012 used the CAS No. 1330-20-7 as the search term with a limit of published articles from 2010 to 2012. A total of 396 articles were identified. This search was refined with:

1330-20-7 and ecotox* resulted in 6 new hits
1330-20-7 and reptil* resulted in no new hits
1330-20-7 and amphib* resulted in no new hits
1330-20-7 and *Xenopus* resulted in no new hits
1330-20-7 and frog resulted in no new hits
1330-20-7 and avian resulted in no new hits
1330-20-7 and mallard resulted in no new hits
1330-20-7 and quail resulted in no new hits
1330-20-7 and bird* resulted in no new hits
1330-20-7 and wild* resulted in 3 new hits
1330-20-7 and mammal* resulted in 4 new hits

Searches of the BIOSIS database, on September 11, 2010, used several keyword combinations to capture articles that might have been missed in the broader searches. These combinations were:

1330-20-7 and ecotox* resulted in 21 hits
1330-20-7 and reptil* resulted in 6 hits
1330-20-7 and amphib* resulted in 7 hits
1330-20-7 and *Xenopus* resulted in 0 hits
1330-20-7 and frog resulted in 1 hits
1330-20-7 and avian resulted in 1 hits
1330-20-7 and mallard resulted in 0 hits
1330-20-7 and quail resulted in 2 hits
1330-20-7 and bird* resulted in 23 hits
1330-20-7 and wildlife resulted in 20 hits
1330-20-7 and toxicity resulted in 252 hits

Additional searches of the BIOSIS database, on September 21, 2012, used a number of keyword combinations with the restriction of 2010 and later to capture articles that might have been missed in the broader searches. These combinations were:

1330-20-7 and ecotox* resulted in one hit
1330-20-7 and reptil* resulted in four hits
1330-20-7 and amphib* resulted in one hit
1330-20-7 and *Xenopus* resulted in no new hits
1330-20-7 and frog resulted in no new hits
1330-20-7 and avian resulted in no new hits
1330-20-7 and mallard resulted in no new hits
1330-20-7 and quail resulted in no new hits
1330-20-7 and bird* resulted in three hits
1330-20-7 and wildlife resulted in one hit
1330-20-7 and toxicity resulted in 28 hits

The different searches defined above identified many of the same articles. Additional references were identified during the review of individual articles. A total of 106 articles were reviewed.

In addition, during the revision and updating of the report on June 25-27, 2018, the original draft was updated with an additional literature search being conducted using the Johns Hopkins Welch Medical Library Multisearch Database.

Using Xylene as a single search term in the title of the document, this search strategy identified 5759 documents with Xylene in the title of the article, in Web of Science; 7370 documents in PubMed; 3 documents in CINAHL Plus; 1757 documents in WorldCat Advanced Search (FirstSearch), of which 39 articles were reviewed; and 1518 documents in Academic Search Complete of which 169 toxicological specific articles were reviewed.

For comprehensive targeted searches, the above specific databases and others (as indicated below) were searched with the aim of refining the identification of specific articles of potential interest. A standard Boolean operator search of PubMed (National Library of Medicine, NIH) of Xylene [TI] as the anchored word in the title with the following search strings were selected using wild-card (*) for optimal returns on search terms and contexts:

Xylene [TI] AND Tox* returned 298 hits
Xylene [TI] AND Tox* refined to 2018 returned 2 hits
Xylene [TI] AND Tox* refined to 2017 returned 6 hits
Xylene [TI] AND Tox* refined to 2016 returned 4 hits
Xylene [TI] AND Tox* refined to 2015 returned 9 hits
Xylene [TI] AND Tox* refined to 2014 returned 11 hits

Species specific search strings yielded the following hits from PubMed:

Xylene [TI] AND mammal returned 481 hits
Xylene [TI] AND animal returned 262 hits
Xylene [TI] AND quail returned 1 hits
Xylene [TI] AND mallard returned 0 hits
Xylene [TI] AND bird returned 2 hits
Xylene [TI] AND avian returned 2 hits
Xylene [TI] AND mouse returned 38 hits
Xylene [TI] AND mice returned 38 hits
Xylene [TI] AND rat returned 180 hits
Xylene [TI] AND wildlife returned 3 hits
Xylene [TI] AND ecotox* returned 11 hits
Xylene [TI] AND amphib* returned 0 hits
Xylene [TI] AND amphibian returned 0 hits
Xylene [TI] AND frog returned 1 hits
Xylene [TI] AND *Xenopus* returned 1 hit
Xylene [TI] AND reptile returned 1 hit
Xylene [TI] AND reptil* returned 0 hits

A repeat search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on June 25-27, 2018, used the CAS No. 1330-20-7 as the search term. A total of 9185 articles were identified. This search was refined with:

1330-20-7 AND ecotox* resulted in 91 hits
1330-20-7 AND reptil* resulted in 1 hits
1330-20-7 AND amphib* resulted in 9 hits
1330-20-7 AND *Xenopus* resulted in three hits
1330-20-7 AND frog resulted in 6 hits
1330-20-7 AND salamander resulted in 0 hits
1330-20-7 AND avian resulted in 3 hits
1330-20-7 AND mallard resulted in 2 hits
1330-20-7 AND quail resulted in 7 hits
1330-20-7 AND bird* resulted in 21 hits
1330-20-7 AND wildlife resulted in 11 hits
1330-20-7 AND mammal* resulted in 262 hits
1330-20-7 AND rat resulted in 1021 hits
1330-20-7 AND mouse resulted in 469 hits

Additional Searches Conducted on Revision of the Report

A repeat search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on August 9, 2019, used the CAS No. 1330-20-7 as the search term and chemical name “Xylene*” with wildcard feature (*) to extract three additional articles that were included in this WTA. Those articles were:

Armenta-Reséndiz M, et al. 2019. Structure-activity Study of Acute Neurobehavioral Effects of Cyclohexane, Benzene, M-Xylene, and Toluene in Rats. *Toxicol Appl Pharmaco*l; 376:38-45.

Duan, W, et al. 2017. Environmental Behavior and Eco-Toxicity of Xylene in Aquatic Environments: A Review. *Ecotoxicol Environ Saf* 145:324-332.

Sutuyeva, LR, et al. 2019. Mortality of Embryos, Developmental Disorders and Changes in Biochemical Parameters in Marsh Frog (Rana Ridibunda) Tadpoles Exposed to the Water-Soluble Fraction of Kazakhstan Crude Oil and O-Xylene. *J Toxicol Environ Health A* 82(3):200-215.