EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Xylene

September 2012

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

AUTHORS AND REVIEWERS

The Office of Environmental Health Hazard Assessment's (OEHHA) Reproductive and Cancer Hazard Assessment Branch was responsible for the preparation of this document.

Primary Authors

Marlissa Campbell, Ph.D.
Staff Toxicologist

Allegra N. Kim, Ph.D.
Research Scientist III

Reproductive Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

OEHHA Reviewers

Allan Hirsch
Chief Deputy Director

Lauren Zeise, Ph.D.
Deputy Director for Scientific Affairs

James M. Donald, Ph.D.
Chief, Reproductive Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Preface

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. It specifies that "a chemical is known to the state to cause reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity ..." The "state's qualified experts" regarding findings of reproductive toxicity are the members of the Developmental and Reproductive Toxicant Identification Committee (DART IC) of the Office of Environmental Health Hazard Assessment (OEHHA) Science Advisory Board[2]. OEHHA, a department within the California Environmental Protection Agency, is the lead agency for implementing Proposition 65.

After consultation with the DART IC, OEHHA selected xylene as a chemical for consideration for listing by the DART IC. The public then was given the opportunity to submit information relevant to the assessment of the evidence on the reproductive toxicity of xylene. One submission was received.

OEHHA developed this document to provide the DART IC with comprehensive information on the reproductive toxicity of xylene for use in its deliberations on whether or not the chemical should be listed under Proposition 65.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code section 25249.5 *et seq*.)
[2] Title 27 Cal. Code of Regs. Section 25302

Acronyms and abbreviations

ASD	autism spectrum disorders
ASPEN	U.S. EPA Assessment System for Population Exposure Nationwide
ATSDR	Agency the Agency for Toxic Substances and Disease Registry
BIA	benzene and its analogues
BPD	biparietal diameter
BTEX	benzene, toluene, ethylbenzene, xylene
BTX	benzene, toluene, xylene
CARB	California Air Resources Board
CI	[95%] confidence interval
CL/P	cleft lip with or without cleft palate
CM	congenital malformation
CNS	central nervous system
CP	cleft palate only
$E_1$3G	estrone 3-glucuronide
EGE	ethylene-based glycol ethers
EPA	Environmental Protection Agency
fab	semiconductor fabrication room
FDR	fecundity density ratio
FIOH	Finnish Institute of Occupational Health
FR	fecundability ratio
GD	gestation day[s]
HAP	hazardous air pollutant
IARC	International Agency for Research on Cancer
IDR	incidence density ratio
IFN-γ	interferon- γ-producing [type 1 T cell]
IL-2	interleukin-2-producing [T cell]
IL-4	interleukin-4-producing [type 2 T cell]
i.p.	intraperitoneal
IQR	interquartile range
IRIS	[U.S. EPA] Integrated Risk Information System
JEM	job-exposure matrix
LDH-C4	lactate dehydrogenase C4
LH	luteinizing hormone
LUR	land use regression, land-use-based regression
mg/m^3	milligrams per cubic meter
mmol/l	millimoles per liter
nBA	n-butyl acetate
NO_2	nitrogen dioxide

NTD	neural tube defect
OR	odds ratio
Pd3G	follicular pregnanediol 3-glucuronide
PDS	photoresist and developer solvents
PND	post-natal day
ppb	parts per billion
ppm	parts per million
REL	reference exposure level
RR	relative risk, risk ratio
SAB	spontaneous abortion
SD	standard deviation
TLV	threshold limit value
TNF-α	tumor necrosis factor-α [producing T cell]
TRI	[U.S. EPA] Toxics Release Inventory
TTP	time to pregnancy
VOC	volatile organic compound
γ-GT	γ[gamma]-glutamyltransferase
$\mu g/m^3$	micrograms per cubic meter

Table of Contents

Preface 3

Acronyms and abbreviations 4

A. Executive Summary 8

B. Introduction 12

B.1. Compound identification and physical properties 12

B.2. Uses and exposure 13

B.2.1. Production and Release of Mixed Xylenes 13

B.2.2. Production and Release of Individual Xylene Isomers 14

B.3. Regulatory Background 15

B.4. Metabolism and Pharmacokinetics 15

B.4.1. Absorption 15

B.4.2. Distribution 16

B.4.3. Metabolism and excretion 17

B.5. Non-DART Toxicities 19

B.5.1. Human data 19

B.5.2. Data from animal studies 20

C. Male Reproductive Toxicology Studies 22

C.1. Human Male Reproductive Studies 22

C.1.1. Human Male Reproductive Studies of Xylene 22

C.1.2. Other Studies Relevant to Male Reproductive Epidemiology of Xylene 26

C.2. Male Animal Reproductive Toxicology Studies 26

C.2.1. Male Animal Reproductive Toxicology Studies of Xylene 26

C.2.2. Related Animal Studies Relevant to Male Reproductive Toxicity of Xylene 28

C.3. Integrative Evaluation for Male Reproductive Toxicity 29

D. Female Reproductive Toxicology Studies 31

D.1. Human Female Reproductive Studies 31

D.1.1. Human Female Reproductive Studies of Xylene 31

D.1.2. Related human female reproductive studies 35

D.2. Female Animal Reproductive Toxicology Studies 36

D.2.1. Information on Female Reproductive Toxicity from Reproductive Toxicity Studies 37

D.2.2. Information on Female Reproductive Toxicity from Developmental Toxicity Studies by the Inhalation Route of Exposure 38

D.2.3. Information on Female Reproductive Toxicity from Developmental Toxicity Studies by the Oral Route of Exposure 41

D.3. Integrative Evaluation for Female Reproductive Toxicity 45

E. Developmental Toxicity Studies 47

E.1. Human Developmental Toxicity Studies 47

E.1.1. Human Developmental Toxicity Studies of Xylene 47

E.1.2. Related Human Developmental Studies 61

E.2. Animal Developmental Toxicity Studies 66

E.2.1. Developmental Toxicity: Technical Xylene by Inhalation 66

E.2.2. Behavioral Endpoints of Prenatal Exposure to Xylenes 70

E.2.3. Developmental Effects of Prenatal Exposures to Individual Xylene Isomers 73

E.2.4. Oral Exposure Studies of Developmental Toxicity in Animals 76

E.2.5. Dermal Exposure Studies of Developmental Toxicity in Animals 76

E.2.6. Related Information of Relevance to the Potential Developmental Toxicity of Xylene 82

E.3. Integrative Evaluation for Developmental Toxicity 83

References 89

Appendix A. Summaries of Epidemiologic Studies 95

Appendix B. Summaries of Animal Studies on Xylene 120

A. Executive Summary

Xylene is a colorless liquid with the molecular formula C_8H_{10}. A flammable, non-explosive aromatic hydrocarbon, xylene consists of a benzene ring with two attached methyl groups, which can vary in position to form the ortho- (o-), meta- (m-), and para- (p-) isomers. Xylene occurs naturally in petroleum and small amounts occur in gasoline and jet fuel. It is used in paints, inks, glues and other products. The population at large is expected to be exposed primarily to mixed xylenes, rather than to any of the xylene isomers individually. The general population can be exposed to xylene via inhalation of indoor and workplace air, automobile exhaust, tobacco smoke, xylene-containing solvents, ingestion of contaminated drinking water, and dermal absorption of xylene-containing products.

Male Reproductive Toxicity

Two human epidemiologic studies of occupational exposure to xylene and four animal studies provide information on the relationship between xylene exposure and male reproductive toxicity. The two epidemiologic studies did not distinguish the potential effects of xylene from those of other concomitant solvent exposures. Reported findings of effects on male reproductive organs are as follows.

- Male reproductive organ weights and function:
    - One cross-sectional epidemiologic study found occupational xylene exposure was associated with an indicator of decreased prostate function.
    - One rat inhalation study reported reduced weights for testis and accessory male reproductive organs, lowered plasma testosterone levels, and lower prostate acid phosphatase activity in xylene-exposed animals. This study was limited by poor methodology such as failing to report the xylene concentration.
    - Two rat inhalation studies found no evidence for adverse effects on testes weights, gross or histological morphology of testis and accessory male reproductive organs, or on hormone levels.
- Semen parameters:
    - The cross-sectional epidemiologic study did not find significant effects of occupational xylene exposure on semen parameters.
    - One animal inhalation study found no association between xylene exposure and sperm count, or the frequency of abnormal sperm morphology.
    - An intraperitoneal (i.p.) injection study found an increased frequency of abnormal sperm with xylene exposure, but only when the animals were housed at temperatures between 24 and 30°C (not at 20 to 24°C).
    - A rat inhalation study reported a decrease in epididymal sperm number for xylene-exposed rats. This study was limited by poor methodology such as failing to report the xylene concentration.

- Fertility and fecundability:
    - A retrospective cohort study found that paternal occupational xylene exposure was not significantly associated with time to pregnancy.
    - The single one-generation reproductive toxicity study conducted by inhalation in rats found no evidence for adverse effects of xylene exposure on mating, fertility, or pregnancy.
    - A small satellite study found no effect on fertility in three male rats following exposure to xylene via inhalation.

Female Reproductive Toxicity

Two epidemiologic studies examined effects of occupational exposure to xylene on female reproductive outcomes. Specifically, one study evaluated time-to-pregnancy, while the second evaluated menstrual patterns. Five additional epidemiologic studies examined maternal exposure to xylene as a potential risk factor for spontaneous abortion (SAB).

The single one-generation reproductive toxicity study of xylene in animals included evaluation of endpoints of female reproductive toxicity such as mating and fertility. No animal studies evaluated potential effects of xylene on the estrous cycle. Additional information relevant to the female reproductive toxicity of xylene comes from 12 developmental toxicity studies conducted in animals. One study considered secretion and circulation of ovarian progesterone and 17ß-estradiol following xylene exposure of pregnant rats.

- Time to pregnancy and fertility:
    - A retrospective cohort epidemiologic study found time to pregnancy was not different in women occupationally exposed to xylene.
    - The rat reproductive toxicity study found no effect of xylene exposure on indices of pregnancy or fertility, or on time-to-mating.
    - Animal developmental toxicity studies that examined the effects of xylene on the numbers of corpora lutea or on implantation frequency found no effects on these endpoints.
- Effects on menstrual cycle and female hormones:
    - One cross-sectional epidemiologic study found low-level exposure to the xylene was associated with oligomenorrhea, although the women were exposed to multiple other industrial chemicals that could have contributed to the observation.
    - An animal study found no effect of exposure to p-xylene on blood flow in the ovary or uterus, or any effect on ovarian progesterone or 17ß-estradiol secretion.
    - The study above also reported decreased peripheral blood levels of progesterone and 17ß-estradiol after 48, but not 24, hours of exposure.
- Embryo-fetal loss:
    - Six epidemiologic studies provide weak to no evidence for exposure to xylene as a risk factor for SAB.

- Three of thirteen animal studies reporting on relevant endpoints provided evidence of xylene-induced embryo-fetal mortality, which tended to be associated with some degree of maternal mortality.

Developmental Toxicity

Nine epidemiologic studies examined the effect of xylene exposure on risk of adverse developmental outcomes. Because exposure to xylene alone was uncommon, the potential effects of xylene were difficult to distinguish from those of other exposures. Fifteen animal studies were identified as having information on the developmental toxicity of technical or mixed xylene and/or its individual isomers.

- Offspring viability:
  - Five epidemiologic studies examined maternal xylene exposure and SAB; of these, two studies found an association but did not adjust for other solvent exposures, and three studies reported no significant association.
  - An epidemiologic study reported no significant association between paternal xylene exposure and SAB.
  - One animal study reported adverse effects of inhaled xylene on offspring viability.
  - An oral study of xylene in mice reported embryo-fetal loss at a level of xylene also associated with greater than 10% maternal mortality.
- Growth:
  - In the two epidemiologic studies that reported on birth weight with xylene exposure, a retrospective cohort study reported increased risk of term low birth weight, and a case control study reported increased birth weight.
  - Animal studies of inhalation exposure to xylene had mixed results for changes in fetal or birth weights.
  - In the one oral study of xylene-induced developmental toxicity in mice, mean fetal weights were significantly reduced in a dose-dependent manner.
- Malformations:
  - Three epidemiologic studies reported no significant association between malformations and maternal xylene exposure, and a fourth study reported no association for paternal xylene exposure.
  - None of the animal studies conducted by the inhalation route of exposure reported significant increases in the frequencies of external or internal soft-tissue malformations or anomalies.
  - Animal studies conducted by the inhalation route of exposure have had mixed findings with respect to increases in the frequencies of skeletal anomalies.
  - An oral study conducted in mice reported significant increases in the frequencies of total malformations with an apparent dose-response relationship, but reported no skeletal effects.

- Developmental neurotoxicity (all inhalation studies):
    - One animal study reported a significant delay in acquisition of the air-righting reflex in rat pups exposed to xylene in utero.
    - Two studies in animals reported effects on the Rotarod test of motor coordination and balance, particularly in female offspring prenatally exposed to xylene.
    - Two studies showed effects on the Morris water maze test, particularly in female offspring prenatally exposed to xylene.
    - One study of prenatal xylene exposure in animals showed no effects on locomotor activity or the acoustic startle response in pups evaluated postnatally.
- Other developmental outcomes:
    - One study in humans found no association between xylene exposure and cord blood cytokine-producing T-cells.

B. Introduction

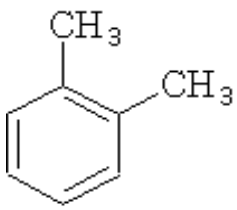
This document reviews the reproductive and developmental toxicity of xylene. It begins with a brief discussion of the physical characteristics and uses of xylene and the resulting exposures. This is followed by sections on each of the major endpoints: male reproductive, female reproductive, and developmental toxicity. Each section reviews effects seen in studies of humans who were exposed to xylene, effects seen in whole animal studies, and other data relevant to reproductive toxicity. The discussion of each endpoint concludes with an integrative evaluation of the entirety of evidence on that endpoint.

B.1. Compound identification and physical properties

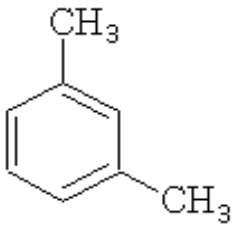
Xylene is a colorless liquid with the molecular formula C_8H_{10}. The molecular weight of xylene is 106.2 daltons. A flammable, non-explosive aromatic hydrocarbon, xylene consists of a benzene ring with two attached methyl groups, which can vary in position to form the ortho- (o-), meta- (m-), and para- (p-) isomers. Xylenes naturally occur in petroleum. The vapor pressure of xylene at 20 °C is 6-16 mmHg. Xylene is relatively insoluble in water: solubility ranges from 100 to 200 parts per million (ppm) at 25°C, depending on the isomer or mix of isomers (Merck, 1989; OEHHA, 1997; ATSDR, 2007).

There are three isomers of xylene. Technical mixtures are sometimes referred to as xylol, or mixed xylene (OEHHA, 1999):

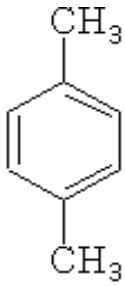
- *technical xylene (o-, m-, p-), xylol*; CAS registry number 1330–20–7
- *o-xylene, ortho-xylene, 1,2-dimethylbenzene, 2-xylene*; CAS registry number 95–47–6
- *m-xylene, meta-xylene, 1,3-dimethylbenzene, 3-xylene*; CAS registry number 108–38–3
- *p-xylene, para-xylene, 1,4-dimethylbenzene, 4-xylene*; CAS registry number 106–42–3



o–xylene



m–xylene



p–xylene

Commercially available xylene typically contains about 40–65% m-xylene and up to 20% each of o-xylene, p-xylene, and ethylbenzene (U.S. EPA, 2003a; ATSDR, 2007).

The exact composition of mixed xylene depends on how it is manufactured. Non-xylene hydrocarbons that may be present, at no more than a fraction of a percentage point, in mixed xylene include: benzene, toluene, trimethylbenzene, phenol, thiophene, and pyridine. Current formulations of mixed xylene are considered to be relatively benzene-free (<0.001%).

B.2. Uses and exposure

Mixed xylene is used heavily in the chemical and petroleum industries, as a solvent and gasoline additive (OEHHA, 1999; ATSDR, 2007). Other uses include xylene as a component of paint thinner and varnishes, as a rubber solvent in the tire industry, as solvents and intermediates in synthetic reactions, and in printing and paper manufacturing. Products using xylene at some stage in the manufacturing process include: polyesters, plasticizers, polyimide polymers, vitamins, pharmaceuticals, and insecticides. Although xylene can enter the environment from natural processes such as forest fires or natural petroleum seeps, most xylene in the atmosphere is from human activity (OEHHA, 1997).

The population at large is expected to be exposed primarily to mixed xylenes, and not to any of the individual xylene isomers on their own (ATSDR, 2007). Xylene and its metabolites have been detected in the general population in samples of human urine, blood, and expired air. The general population is believed to be exposed to xylene via inhalation of indoor and workplace air, inhalation of automobile exhaust, ingestion of contaminated drinking water, smoking, and inhalation or dermal absorption of xylene-containing solvents.

B.2.1. Production and Release of Mixed Xylenes

Production capacity of mixed xylene in the U.S. has been estimated at approximately 18 billion pounds (ATSDR, 2007). Based on data from the U.S. Environmental Protection Agency’s (U.S. EPA's) Toxics Release Inventory (TRI), the Agency for Toxic Substances and Disease Registry (ATSDR, 2007) reported that total production of mixed xylene reported by U.S. manufacturers has exceeded 1 billion pounds for each reporting year up to and including 2002.

ATSDR (2007) tabulated TRI data from 2004 (released in 2006) that show the number of facilities in each state, including California, which manufactured, processed, or used mixed xylene in the year 2004. For California, 307 facilities were required to report. Activities and uses reported by these facilities included:

- production
- importation
- on-site use and processing
- sale and distribution
- manufacturing byproduct or impurity
- reactant

- formulation component
- chemical processing aid
- manufacturing aid

For the reporting year 2009, TRI data from U.S. EPA (2010) indicate that a total of 833,729.23 pounds of xylene were "disposed of or otherwise released" in California. ATSDR's (2007) review of TRI data from earlier years showed that 93% of xylene releases in California were to air; an additional 5% of releases were to land, with the remainder either to water or classified as "other."

Fugitive emissions of xylenes to the atmosphere can occur from industrial sources, automobile exhaust, and volatilization of solvents (ATSDR, 2007). Xylenes discharged to soil or surface water are expected to volatilize into the atmosphere. California outdoor air monitoring data from 2011 showed statewide means for (m+p)-xylene of 0.40 parts per billion (ppb) (California Air Resources Board [CARB], 2012a) and 0.15 ppb for o-xylene (CARB, 2012b). Data on multiple air toxics, including (m+p)-xylene and o-xylene, were collected from fixed sites at 10 locations in the greater Los Angeles area over a two-year period (South Coast Air Quality Management District, 2008). Over the study period, levels of (m+p)-xylene for all locations ranged from a low of 0.62 ppb to a high of 1.44 ppb. Levels of o-xylene ranged from 0.15 to 0.40 ppb.

Xylenes can leach into groundwater, though groundwater surveys have found xylene in less than 5% of U.S. groundwater (ATSDR, 2007). For groundwater that is contaminated, concentrations of xylene as high as 10,000 ppb have been reported. Among samples of drinking water in the U.S., fewer than 6% of samples collected contained xylenes. For those positive samples, xylene concentrations were typically less than 2 ppb. Xylene can also be present in food, with a variety of foodstuffs showing typical concentrations ranging from 1 to 100 ppb. Bioaccumulation is considered to be "very modest" (<100), and bio-magnification via the food chain has not been observed.

B.2.2. Production and Release of Individual Xylene Isomers

TRI data on production and use of individual xylene isomers are reported separately from the mixed formulations; it is not clear how much overlap occurs for quantities of isomers reported singly and/or as part of a mixture (ATSDR, 2007). Individual isomers are used in the manufacture of some polymers.

Of the three isomers, p-xylene is produced in the highest quantities in the U.S. (OEHHA, 1999). California emissions data for 1996 reported under the Air Toxics Hot Spots Act included quantities of specific xylene isomers: 51,203 pounds of p-xylene, 34,573 pounds of o-xylene, and 30,440 pounds of m-xylene.

Table 1. Estimated U.S. Annual Xylene Production Capacity

	Mixed xylene	m-xylene	o-xylene	p-xylene
Total annual capacity in millions of pounds	18,209	Not reported	976	10,903

(ATSDR, 2007)

According to information summarized by ATSDR (2007), m-xylene is the most prevalent isomer found in the environment. People who work at or live near locations where individual xylene isomers are used in large quantities may have higher exposures to one xylene isomer as compared to other isomers. It is difficult to separate the m- and p-isomers of xylene from one another when analyzing biological and environmental samples.

B.3. Regulatory Background

OEHHA (2008) has established 8-hour acute and chronic reference exposure levels (RELs) for mixed xylenes. The acute REL is 22,000 micrograms per cubic meter (µg/m³), and the chronic REL is 700 µg/m^3, or 200 ppb. For both values, the critical effects are eye irritation and toxicity to the respiratory and nervous systems. California's Public Health Goal (PHG) for xylene(s) in drinking water is 1.8 ppm (OEHHA, 1997). The State's Maximum Contaminant Level (MCL) for xylene in drinking water is 1.75 ppm, based on non-cancer effects from a chronic rat study.

U.S. EPA (2003b) has established a reference dose (RfD) for oral exposure to mixed xylenes of 0.2 milligrams per kilogram-day (mg/kg-day). The critical effects were considered to be decreased body weight and increased mortality in a chronic oral rat study. The inhalation reference concentration (RfC) has been established at 0.1 milligrams per cubic meter (mg/m^3), based on impaired motor coordination (decreased rotarod performance) observed in a subchronic inhalation study in male rats.

The U.S. EPA Integrated Risk Information System (IRIS) entry for xylenes (U.S. EPA, 2003b) characterized both human and animal carcinogenicity data as "inadequate." The International Agency for Research on Cancer (IARC; 1989) similarly concluded that xylene is "not classifiable as to its carcinogenicity to humans."

B.4. Metabolism and Pharmacokinetics

B.4.1. Absorption

Xylene is well absorbed by both the oral and inhalation routes of exposure (U.S. EPA, 2003a; OEHHA, 1997). Absorption efficiencies have been estimated at 90% for the oral route, and 60-65% for xylene and each of its isomers by inhalation. Given consistent absorption efficiencies by the inhalation route of exposure, the factors controlling total xylene uptake appeared to be ambient concentrations and the subjects' ventilation rates.

Experiments involving gavage administration of radiolabeled m-xylene to male and female rats indicated rapid absorption with peak plasma concentrations observed within 20 minutes post-dosing (U.S. EPA, 2003a). Mean absorption half-time in female rats was significantly shorter than in males (0.31 and 0.64 hours, respectively). As described below in section B.4.3, there was also a difference in elimination half-time between females and males suggesting a gender-dependent difference in xylene pharmacokinetics for these animals.

Efficiency of absorption by the dermal route depends upon exposure conditions, but is generally estimated to be less than 1% (OEHHA, 1997). Dermal absorption of xylene from vapor in the air has been estimated at 0.1-0.2% of what would be absorbed by inhalation. A dermal absorption rate coefficient of 0.08 centimeter per hour (cm/hr) has been estimated for the rate of dermal absorption of xylene in water.

B.4.2. Distribution

B.4.2.1. General xylene distribution

As compared to organic solvents in general, xylene is considered to be relatively soluble in blood (OEHHA, 1997). It distributes rapidly throughout the body, with a particular affinity for tissues rich in lipids such as body fat, liver, and the brain (U.S. EPA, 2003a).

Adipose tissue has the highest affinity for xylene of all tissues. Xylene sequestered in adipose tissue is expected to have the lowest rate of metabolism in the body, the slowest movement back to blood, and the longest persistence. As summarized by U.S. EPA (2003a), experiments in rats found 10-20% of the administered xylene was distributed to adipose tissue. Following the end of a week-long exposure period, the xylene concentration in gluteal subcutaneous fat was 10-fold higher than that in venous blood.

With inhalation exposures of less than one hour duration, concentrations do not reach equilibrium, and most of the xylene is gone within 60 to 90 minutes (OEHHA, 1997). Longer exposures lead to more fat storage of the total xylene body load, and slower, second phase elimination becomes significant.

B.4.2.2. Xylene distribution in pregnant animals and fetuses

The distribution of ^{14}C-xylene was studied in pregnant mice exposed by inhalation (Ghantous and Danielsson, 1986). Whole-animal autoradiography and liquid scintillation were used to distinguish between volatile, water-soluble, and tissue-bound radiolabel.

Immediately after inhalation, xylene reached high concentrations in lipid-rich maternal tissues such as brain and fat. Initially high levels were also found in well perfused organs, such as liver and kidney. Rapid elimination resulted in low concentrations at one-hour post-exposure in all maternal tissues other than fat. Metabolites reached peak levels between 30 minutes and one hour after inhalation; these were also eliminated

relatively rapidly. Water-soluble metabolites were found to accumulate in the nasal mucosa and olfactory bulb when measured at four and 24 hours post-exposure. Volatile radioactivity was identified in placenta and fetuses almost immediately, and up to one hour post-inhalation of xylene. Fetal levels were much lower than those found in maternal tissues. Labeled xylene metabolites tended to concentrate in embryonic neuroepithelium during early gestation.

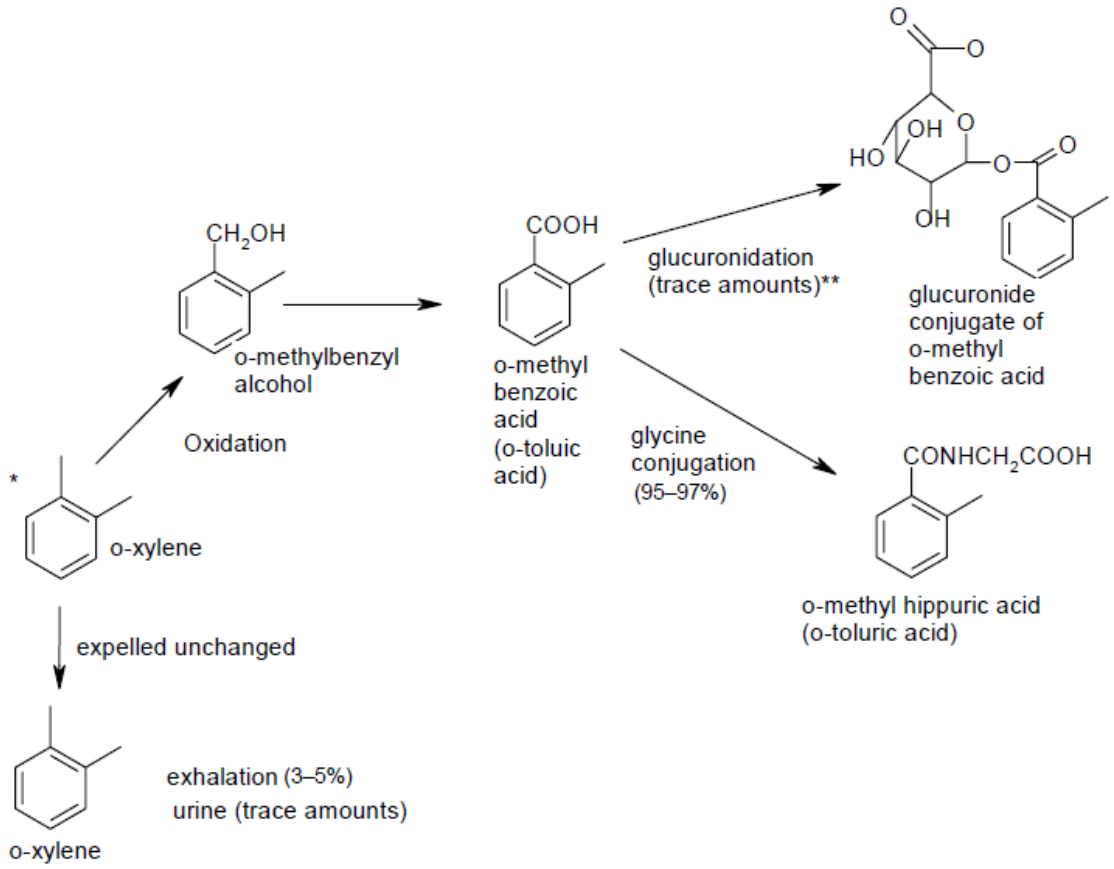
Late gestation fetal brains showed lower concentrations of radiolabeled xylene metabolites as compared to other fetal organs. One and four hours post-dosing of dams, spots of radioactivity in the fetal urinary tract were taken to indicate active elimination by the fetus. At later time points, and 24 hours post-dosing, label was concentrated in the uterine fluid, as opposed to chorioallantoic placenta, fetal tissues, or amniotic fluid. The lack of tissue-bound metabolites in fetal tissues during late gestation was taken to indicate an inability of fetal tissues to form reactive metabolites.

B.4.3. Metabolism and excretion

Xylene is primarily metabolized in the liver, with some activity occurring in the lung and kidneys (U.S. EPA, 2003a; OEHHA, 1997). When studied using human liver microsomes, methyl hydroxylation to methyl benzyl alcohol was found to be the major mechanism of xylene metabolism. This reaction is catalyzed by cytochrome P_{450}-2E1, a metabolic process which is both saturable and inducible.

As summarized by OEHHA (1997), oxidation of the alcohol moiety forms methyl benzoic acid (toluic acid), which in turn is either conjugated with glycine (to methylhippuric acid) or glucuronic acid (to methylbenzyl glucturonide). These conjugates are excreted in urine, along with minor metabolites such as methylbenzyl mercapturic acid and dimethyl phenol. Different animal species show varying proportions of these metabolites (IARC, 1989).

Figure 1. Proposed metabolic pathways for o-xylene, as a model for all xylene isomers (U.S. EPA, 2003a)



Disappearance of xylene from the blood of humans exposed by inhalation has been shown to follow biphasic, first-order kinetics with half-lives of about 0.5-1 hour and 20-30 hours (U.S. EPA, 2003a). For monitoring purposes, measurements of exhaled xylene are considered of limited usefulness, as blood and breath levels drop quickly as soon as exposure ceases (OEHHA, 1997). Urinary methylhippuric acid content is considered a useful surrogate for xylene exposure.

Experiments involving gavage administration of radiolabeled m-xylene to male and female rats indicated elimination half-time was longer in females than in males (11.42 and 6.77 hours, respectively) (U.S. EPA, 2003a). This difference contributes to an overall gender-dependent difference in xylene pharmacokinetics for these animals, although the total amount of radioactivity excreted in urine over 48 hours did not differ between the sexes. Expired air was a secondary route of elimination for ingested radiolabeled parent compound, accounting for 14-22% of the administered dose in females and 9-20% in males.

B.5. Non-DART Toxicities

B.5.1. Human data

B.5.1.1. Acute toxicity in humans

Similar to other organic solvents, acute inhalation exposure to xylene has readily observable neurological effects: mild excitation at a low dose, followed by sedation and narcosis with higher exposures (OEHHA, 1997). Technical xylene and each of the three xylene isomers have essentially identical effects, with comparable dose-response ranges, insofar as these have been characterized. Approximate xylene exposure ranges for specific effects in humans are as follows (U.S. EPA, 2003a; OEHHA, 1997):

- Odor threshold — 1 ppm in air
- Taste threshold — 1 ppm in water
- 1-100 ppm — nausea, headache, irritation of mucous membranes
- $\geq$ 100 ppm — sedation, disorientation, ataxia
- At several thousand ppm — severe lung irritation leading to pulmonary inflammation, edema, and hemorrhage; deaths are attributed to respiratory depression, rather than lung damage per se

Most of the data associating xylene with neurological impairment in humans come from case reports following exposures in the range of 800-10,000 ppm (U.S. EPA, 2003a). Based on controlled exposure experiments in humans, xylene concentrations of approximately 100-200 ppm appear to be the threshold level for short-term reversible neurological and irritation effects.

In general, the more severe acute effects of xylene inhalation are thought to involve molecular interactions of the parent solvent, rather than metabolites, with membranes of the affected tissues (U.S. EPA, 2003a). Such effects are most pronounced at exposures in excess of 1,000 ppm.

B.5.1.2. Repeated dose toxicity and carcinogenicity in humans

According to U.S. EPA (2003a), available human data do not provide adequate evidence for neurological impairment resulting from repeated exposure to xylene at concentrations $\leq$ 200 ppm.

Human epidemiological studies have not provided clear evidence on the carcinogenicity or non-carcinogenicity of xylene by inhalation (OEHHA, 1997). At least part of the lack of clarity is thought to be due to the fact that long-term occupational exposures typically involve many different solvents, making it difficult to isolate an effect of any single compound. As noted above (Section B.3), U.S. EPA characterizes human carcinogenicity data as "inadequate" and IARC considers xylene "not classifiable as to its carcinogenicity to humans".

B.5.2. Data from animal studies

B.5.2.1. Acute toxicity in animals

Experimental animals have shown effects from acute exposures to xylene that are similar to those described for humans (OEHHA, 1997). Observations included: acute excitation followed by sedation, ataxia, altered visual and auditory evoked potentials, muscle spasms, and labored breathing. Acute xylene doses causing these behavioral effects seem to be similar to those effective in humans.

As summarized by OEHHA (1997):

- Four-hour inhalation LC_{50} in rats, 6,500 ppm
- Four-hour inhalation LC_{50} in mice, 4,000-5,000 ppm
- Gavage LD_{50} in rats, 3.5 g/kg
- Gavage LD_{50} in mice, 5.5 g/kg

Because xylene solubility in water is limited to 200 ppm, there are no drinking-water toxicity studies of xylene in animals (OEHHA, 1997).

B.5.2.2. Repeated dose toxicity, and carcinogenicity in animals

In similarity to certain other solvents, short-term inhalation of a high concentration of xylene (1,450 ppm, eight hours) or longer-term inhalation of a lower concentration (800 ppm, 14 hrs/day, six weeks) has been associated with hearing loss in rats (U.S. EPA, 2003; OEHHA, 1997). Hearing loss was also reported in rats exposed to 1,000 ppm xylene for 18 hours per day, seven days per week, for 61 days. This effect appears to be permanent, and selective for mid-frequency sounds.

As summarized and evaluated by U.S. EPA (2003), subchronic studies in animals provide evidence for neurological effects of xylene following repeated inhalation exposures. Statistically significant effects have been observed on neurobehavioral tests such as rotarod performance, spontaneous motor activity, and radial maze performance when testing was conducted at least 24 hours following the last xylene exposure. These types of effects were seen in several studies of rats exposed to 100 ppm m-xylene, six hours per day, five days per week, for three months.

OEHHA (1997) summarized findings from chronic toxicity studies of xylene carried out by the National Toxicology Program (NTP) in rats and mice. Both species of animals were dosed by gavage with xylene administered in corn oil, five days/week for 103 weeks. Evidence of moderate toxicity included a slight decrease in body weight of male rats at the high dose of 500 mg/kg-day, and hyperactivity in high-dose group mice (1,000 mg/kg-day). No increases in neoplastic or non-neoplastic lesions were considered to be related to xylene exposure in either species.

Decreased body weight appears to be a general effect of repeat-exposure studies (U.S. EPA, 2003). According to U.S. EPA's summary (2003), effects on body weight in rats and mice had generally been reported with oral doses of xylene in the range of 500-800

mg/kg-day, or higher. In discussing the NTP study mentioned above, however, U.S. EPA notes that no effect on body weight was seen in female rats (high-dose, 500 mg/kg-day) or male or female mice (high-dose, 1000 mg/kg-day).

Other noncancer effects reported in various oral repeated dose-studies include (U.S. EPA, 2003):

- Increased liver weights
- Increased kidney weights with associated nephropathy
- Gross clinical signs and symptoms of neurological impairment

These effects, however, were only observed in single studies and not reproduced across the board (U.S. EPA, 2003). Where reported, such effects apparently occurred at higher doses than the levels inducing body weight changes. Neurological data, in particular, are limited by a lack of comprehensive evaluations for any persistent changes in neurobehavioral endpoints, such as a functional observational battery following oral exposures to xylene.

Neither *in vivo* nor *in vitro* mutagenicity studies have provided evidence for a genotoxic effect of xylene (OEHHA, 1997). Carcinogenicity assessments have been deemed to provide insufficient evidence for carcinogenicity of xylene in animals (IARC, 1989; U.S. EPA, 2003a).

C. Male Reproductive Toxicology Studies

C.1. Human Male Reproductive Studies

Two epidemiologic studies examined associations between male reproductive outcomes and exposure to xylene and other organic solvents (Xiao et al., 1999 and 2001 and Sallmén et al., 1998). These studies are summarized in Table 2 and in greater detail in Appendix A. In Table 2 and subsequent tables summarizing epidemiologic studies, statistically significant findings are indicated by boldface type.

Two additional epidemiologic studies examined the relationship between sperm or semen and exposure to groups of compounds that include xylene, but did not report results for xylene specifically (De Celis et al., 2000 and Lemasters et al., 1999). These studies are described in Section C.1.2.

C.1.1. Human Male Reproductive Studies of Xylene

Xiao et al. conducted a cross-sectional study in Zhejiang Province, China, and reported on this study in two separate articles (Xiao et al., 1999 and Xiao et al., 2001). The sample included 24 exposed men working in shoemaking, spray painting, or paint manufacturing, and 37 managers, who were considered unexposed. Blood and semen samples were analyzed for benzene, toluene, and xylene (BTX), and semen was analyzed for indicators of effects on semen and accessory sex gland function. Xiao et al. report that blood xylene was statistically significantly associated with decreased seminal γ-glutamyltransferase (γ-GT) activity ($p=0.0002$), indicating poorer prostate function. However, the interpretation of the reported statistics (Beta=-30.2810, B=7.5835, and Constant=-0.4086) is unclear, and the analysis does not appear to adjust for exposure to other solvents. Semen benzene was also associated with decreased γ-GT activity. Solvent exposure (as defined by occupation) was significantly associated with several parameters, including lower sperm activity, lower acrosin activity, lower γ-GT activity, and lower lactose dehydrogenase C4 activity. The authors note that BTX are used mostly in mixed form. The reports do not provide some important details, such as subject selection and recruitment, variables in the statistical models, and the interpretation of the statistics.

A study by Sallmén et al. (1998) investigated whether paternal exposure to organic solvents (styrene, toluene, xylene, tetracholoroethylene, tricholoroethylene, and 1,1,1-trichloroethane) is associated with delayed conception. For this time to pregnancy (TTP) study, the cohort of exposed and unexposed subjects comprised cases and controls from a previous study of spontaneous abortion (SAB) and congenital malformations (Taskinen et al., 1989). The source population was male workers who were biomonitored for organic solvent exposure from 1965 through 1983. Each husband's occupational exposure to solvents during the calendar year in which the pregnancy had started was assessed based on self-reported occupation, job description, and solvent or other chemical usage, as well as biomonitoring data

(available for 69% of the subjects), from the previous study. The same exposure assessment was adopted for the present study if the husband had the same job at the beginning of the TTP as 80 days (estimated period of spermatogenesis) before the beginning of the study pregnancy. TTP and related information were collected by questionnaires mailed to the wives six years after the first study, eight to 18 years after the pregnancies ended. The sample included 282 couples, with 70 men in the low/intermediate xylene exposure category and 51 in the high category. Data were analyzed using discrete proportional hazards regression, with fecundability density ratio (FDR; [fecundability of the exposed]/[fecundability of the unexposed]) as the outcome. An FDR less than unity indicates reduced probability of conception. The FDRs (95% confidence interval [CI]) for xylene were 0.75 (0.52-1.09) for low/intermediate exposure and 0.91 (0.61-1.36) for high/frequent exposure, adjusted for short menstrual cycle, long or irregular menstrual cycle, older age at menarche, frequency of intercourse, maternal age, maternal exposure to organic solvents, and a variable for missing information. The authors note that simultaneous exposure to multiple solvents was common and risks cannot be attributed to specific solvents.

Table 2. Human Male Reproductive Studies

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Xiao et al., 1999 and Xiao et al., 2001 Zhejiang Province, China June 1994-July 1996 Objective: to examine the effects of benzene, toluene, and xylene (BTX) on semen quality of workers in shoemaking, spray painting, or paint manufacturing	Semen samples taken from 24 of 56 married workers who had ≥ 1 child and were exposed to high concentrations of airborne BTX and 37 of 40 "age-occupational-matched non-exposed" managers Semen analyses included liquefaction time, semen pH, sperm concentration, total sperm count, percentage vitality, sperm activity, acrosin activity, seminal fructose, seminal γ-glutamyltransferase (γ-GT) activity, relative activity of lactose dehydrogenase C4 (LDH-C4). Chi-squared and t tests, multiple regression analysis	Mean work air concentrations were: xylene 8.21 mg/m^3; benzene 103.34 mg/m^3; toluene 42.73 mg/m^3. Methods for measuring these concentrations were not described. Blood and semen samples were analyzed for BTX using headspace chromatography	Subjects were interviewed about reproductive, medical, and occupational history; tobacco and alcohol use.	**Blood xylene was associated with decreased γ-GT activity** (reflecting prostate function)**. However, the interpretation of the reported statistics (Beta=-30.2810, B=7.5835, and Constant=-0.4086) is unclear.** Whether the association for xylene was independent of effects of other solvents was not reported. Semen benzene was also associated with γ-GT activity. Sperm activity, acrosin activity, γ-GT activity, LDH-C4 activity, and sperm vitality were significantly lower in BTX-exposed workers (authors' tables do not indicate significance for sperm vitality).	The study is not reported in detail. Unexposed workers were selected from management and had a much higher participation rate, and differed slightly from the exposed in terms of age, drinking, smoking, and other characteristics. If BTX reduces fertility, selection of men with children could bias results by excluding those whose fertility was adversely affected by exposure. Less than half of blood and semen samples among those classified as exposed had detectable BTX. The authors point out that workers were typically exposed to BTX mixtures.

Table 2. Human Male Reproductive Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Sallmén et al., 1998 Finland Pregnancies between 1973-1983 Objective: To investigate whether paternal exposure to organic solvents is associated with delayed conception This study is an extension of a case-control study of effects of paternal exposure to organic solvents on SAB and congenital malformations (Taskinen et al., 1989)	Retrospective cohort (convenience sample) Male workers had participated in the previous study and were biomonitored for solvent exposure from 1965-1983 and. 282 couples were included. Questionnaires were mailed to wives 8-18 years after the pregnancies to collect information about TTP and related questions. Discrete proportional hazards regression; outcome is fecundability density ratio (FDR) = (fecundability of the exposed) ÷ (fecundability of the unexposed).	Paternal exposure was generally based on self-reported information about occupation and chemical use during the year the pregnancy started, and biomonitoring data (available for 69%). Likelihood of exposure was categorized as: • unexposed, • potentially exposed, or • likely exposed. Exposure levels were: • high or frequent (daily handling of solvents, or biomonitoring indicates exposure > reference values for the general population) • intermediate or low (exposure 1-4 days/week and biomonitoring indicates low exposure) • none	Information from husbands included frequency of exposure to gases, vapors, dusts, fumes, and other chemicals, or radiation in the workplace, earlier employment, chronic diseases, smoking, and alcohol consumption. Women were asked about recent oral contraceptive or intrauterine device use, unplanned pregnancy, frequency of intercourse, breast feeding, caffeine intake, year of pregnancy, occupational and lifestyle exposures, and conditions that could affect husband's fertility. Pregnancy history and other related data had been collected for the previous study.	FDRs (CI) for xylene, adjusted for age, older age at menarche, menstrual cycle variables, frequency of intercourse, maternal and paternal smoking, maternal exposure to organic solvents, year of pregnancy, and missing information: • low/intermediate exposure (n=70), FDR=0.75 (0.52-1.09) • high/frequent exposure (n=51), FDR=0.91 (0.61-1.36) Results suggest xylene exposure might be associated with reduced incidence of pregnancy, though the observed effect was smaller at the higher exposure level, and not significant.	Simultaneous exposure to multiple solvents was common. 11 men in the high xylene exposure group and 2 men in the low/ intermediate group had biological measurements. The reported median urinary methylhippuric acid levels were 0.36 mmol/l in the high exposure group and 0 mmol/l in the low/intermediate group. 23% of pregnancies ended in SAB. The authors note that such pregnancies tend to have longer TTP than those ending in births and TTP data might be less accurate for SABs than for births. If solvent exposure is associated with sterility or long TTP, a bias toward the null would result (by including only couples who achieved pregnancy).

C.1.2. Other Studies Relevant to Male Reproductive Epidemiology of Xylene

In a cross-sectional study, De Celis et al. (2000) examined sperm and semen parameters in men working at a rubber factory in Mexico City. This study included measurements of xylene and other hydrocarbons throughout the workday, and semen samples were collected weekly for three weeks. Exposure levels were high, e.g., air concentrations of xylene were 10,000-12,000 ppb. Most exposed workers experienced symptoms, such as headache, forgetfulness, and depression. The unexposed administrative workers were more likely to have normospermia (odds ratio [OR]=16.0, 95% CI 5.1-52.0), higher mean sperm counts, percentage motile sperm, and percentage normal sperm forms. Samples from exposed men had a higher mean percentage of nonspecific aggregation and were less likely to have normal viscosity and complete liquefaction.

Lemasters et al. (1999) conducted a prospective, repeated measures examination of jet fuels and solvent mixtures (including xylene) in relation to semen parameters in aircraft maintenance workers. Semen samples were taken before exposure began, and 15 and 30 weeks later, during occupational exposure. Due to low exposure levels and the fact that most men were exposed to multiple solvents, the authors summed the concentrations of xylenes; 1,1,1-trichloroethane; toluene; methyl ethyl ketone; and methylene chloride. The mean total solvent exposure level for all workers was 1.6 ppb. Exposure as measured by total solvent level was not significantly associated with changes in semen parameters.

C.2. Male Animal Reproductive Toxicology Studies

Four studies provided data on the male reproductive toxicity of xylene in animals (Biodynamics, 1983; Nylen et al., 1989; Washington et al., 1983; Yamada, 1993). Three of these studies were conducted by the inhalation route of exposure, and one by i.p. injection. No studies on the reproductive toxicity of xylene following oral exposure were available.

An additional study (Chung et al., 1999) reported on testicular effects of a solvent mixture including xylene.

C.2.1. Male Animal Reproductive Toxicology Studies of Xylene

In a large one-generation reproductive toxicity study, male and female CD rats were exposed to mixed xylenes by inhalation for 131 days pre-mating, and 20 days of mating (Biodynamics, 1983). Animals were exposed daily for six hours per day to xylene concentrations of 0, 60, 250, or 500 ppm.

No clinical symptoms of toxicity were reported for the treatment period. No mortality was observed among xylene-exposed males. One control male died during the study. No adverse effects were found on body weights or the weights of male reproductive organs. No abnormal findings were reported upon gross postmortem evaluation. No

differences were found among groups in pregnancy or fertility indices. The mating index was not affected for males in any of the treatment groups. Variation among groups in time-to-mating could not be attributed to treatment.

The limited data available for F1 males on post-natal day (PND)-21 and PND-49 did not provide evidence for an effect of xylene on testes weights.

Nylen et al. (1989) exposed six male Sprague-Dawley rats to 1,000 ppm xylene by inhalation. Animals were exposed for 18 hours per day, seven days per week, for 61 days. Evaluations were made at two weeks, 10 months, and 14 months following cessation of treatment.

Evaluations included gross and histological pathology of testis and accessory male reproductive organs, hormone levels, sperm counts and morphology, and fertility (in three of six xylene-treated rats). The data provided no evidence for an adverse effect of xylene, under the experimental conditions tested, on the male reproductive system.

Yamada (1993) exposed male Wistar rats, seven to eight weeks of age, to xylene by inhalation. Animals were placed in the inhalation apparatus twice per day for seven days, with sacrifice for evaluation of male reproductive organs performed on the day following the seventh day of exposure. Xylene concentration was not measured directly, but animals were left in the apparatus until the righting reflex disappeared. Hence, the neurological effect (loss of righting reflex) indicates that the animals were exposed to a biologically significant concentration.

Animals were weighed daily during the treatment period; xylene-treated animals showed a significant difference from controls only on the last day of treatment ($P < 0.05$).

Under the conditions of this study, xylene exposure was associated with reduced weights of testes and accessory male reproductive organs. Lowered plasma testosterone levels were also observed, as well as a reduction in prostate acid phosphatase activity. There was also a reduction in the numbers of epididymal sperm.

In the one available male reproductive toxicity of xylene not conducted by the inhalation route of exposure, Washington et al. (1983) gave o-xylene to Sprague Dawley rats by i.p. injection. Controls were given the corn oil vehicle alone, while doses for the treated animals were 0.5 or 1.5 milliliters per kilogram of body weight (ml/kg-bw). Treatments were given over a period of two days. The number of animals per group is not stated in the report.

Five weeks following the end of treatment, sperm morphology was evaluated by light microscopy. Treated animals kept at an ambient temperature of 20-24°C showed no significant increase in the frequency of abnormal sperm over levels seen among control animals. When housed at temperatures between 24 and 30°C, however, rats given 0.5 ml/kg o-xylene showed a significant increase in abnormal sperm of 1.23% over controls ($p < 0.05$). Untreated controls showed no effect of temperature on sperm morphology.

Table 3. Animal Studies of Male Reproductive Toxicity

Reference	Study design	Systemic toxicity	Male reproductive toxicity
Biodynamics (1983)	1-generation study; CD rats; N=10-30; 0, 60, 250, and 500 ppm; inhalation; daily, 6 hr/day; 131-days pre-mating, 20 day mating period	No mortality No clinical symptoms of toxicity No effect on body weight No gross pathology	No effect on testes weights No effects on mating or fertility
Nylen et al. (1989)	♂ Sprague-Dawley rats; N = 6; inhalation, 0, 1000 ppm; 8 hr/day, 7 days/wk, 61 days Evaluations at 2 wks, and 10 and 14 months after the end of treatment Fertility test at 13 months after treatment; N = 3	Not noted	No gross or histological changes in testis or epididymis No change in testes or prostate weights No change in sperm morphology No change in testosterone levels or other hormonal endpoints No effects on fertility
Washington et al. (1983)	♂Sprague Dawley rats, 10-16 wks old; 0.5 or 1.5 ml/kg bw o-xylene in corn oil, by i.p. injection. Corn oil controls N not provided 2-day treatment Sperm morphology evaluated at 5 wks post-treatment Epididymal sperm evaluated by light microscopy	Not noted	↑ Abnormal sperm with 0.5 ml/kg o-xylene ($p < 0.05$) only at room temp 24-30°C. No effect at room temp 20-24°C
Yamada (1993)	♂ Wistar rats; N = 5; 7-8 wks old; xylene by inhalation, 2X per day, 7 days Concentration not measured, animals exposed until they lost the righting reflex	↓ Body weight on treatment day seven	↓ Testis weight ↓ Epididymis weight ↓ Prostate weight ↓ Weight of vas deferens and seminal vesicles ↓ Plasma testosterone ↓ Prostate acid phosphatase activity ↓ Epididymal sperm

C.2.2. Related Animal Studies Relevant to Male Reproductive Toxicity of Xylene

Chung et al. (1999) conducted a rat study to investigate the testicular effects of a solvent mixture to which human painters are commonly exposed. The solvent mixture contained ethylene glycol monomethyl ether (EGE), toluene, and xylene. Male rats were exposed to EGE (200 mg/kg) alone, or in combination with toluene (250 mg/kg) and xylene (500 mg/kg).

Male Sprague-Dawley rats, 250-300 grams (g), were given solvents by daily gavage in an olive oil vehicle. Preliminary experiments were conducted using multiple doses of EGE alone, or toluene and xylene in combination. Xylene was not tested independently, nor were results from the dose range-finding studies provided.

After four weeks of treatment with the test doses above, animals exposed to EGE alone showed a 25% greater extent of testicular atrophy than those animals exposed to the combination treatment. The authors attribute their findings to interference by toluene and xylene with production of the toxic EGE metabolite, ethoxyacetic acid (EAA). Data on plasma concentrations of EAA during these experiments were taken to support this interpretation.

C.3. Integrative Evaluation for Male Reproductive Toxicity

There are two human epidemiologic studies of male occupational exposure to xylene (Xiao et al., 1999 and 2001 and Sallmén et al., 1998), and four animal studies of xylene exposure, three by inhalation (Biodynamics, 1983; Nylen et al., 1989; Yamada, 1993) and one by i.p. injection (Washington et al., 1983). Effects on male reproductive organs (including indicators of organ function, hormone levels, and gross and histological pathology), semen parameters, as well as fertility and mating, have been evaluated.

One epidemiologic study found occupational xylene exposure was associated with decreased seminal γ-GT activity (reflecting prostate function) (Xiao et al., 1999 and 2001). While no animal studies have considered this specific endpoint, there are some animal data on male reproductive organ weights and function. One rat inhalation study (Yamada, 1993) reported reduced weights for testis and accessory male reproductive organs in a small group of xylene-exposed animals. This study also reported lowered plasma testosterone levels, and lower prostate acid phosphatase activity in treated animals. Confidence in these data is severely limited by the lack of quantitative exposure information, as well as small group size and a lack of information on toxicity to other organs. Two better conducted rat studies found no evidence for an adverse effect on testes weights (Biodynamics, 1983), on gross or histological morphology of testis and accessory male reproductive organs, or on hormone levels (Nylen et al., 1989).

Studies that examined semen parameters also had mixed findings. Xiao et al. (1999 and 2001) did not find significant effects of occupational xylene exposure on semen parameters in humans. Of three animal studies considering sperm parameters, the best conducted observed no associations between xylene exposure and sperm counts and morphology in rats (Nylen et al., 1989). A study conducted by the i.p. injection route of exposure in rats (Washington et al., 1983) found that xylene-exposed animals also subjected to housing at temperatures between 24 and 30°C showed an increase in the frequency of abnormal sperm, while xylene-treated animals kept between 20 and 24°C showed no effect. Yamada (1993) reported a decrease in epididymal sperm number for xylene-exposed rats; the limitations of that study are discussed above.

An epidemiologic study of fecundability found that paternal occupational xylene exposure was not significantly associated with TTP (Sallmén et al., 1998). Rat studies found no evidence for an adverse effect on fertility (Nylen et al., 1989) or pregnancy, fertility, or mating indices (Biodynamics, 1983).

Related studies showed effects of high exposure to mixtures that include xylene on human sperm (De Celis et al., 2000) and evidence for interaction among xylene, toluene and EGE exposures in rats, where the presence of xylene and toluene in the solvent mixture containing ethylene glycol monomethyl ether (EGE) appeared to attenuate adverse effects observed with EGE alone (Chung et al., 1999).

For each type of male reproductive outcome, the epidemiologic evidence for effects of xylene is sparse. The two studies did not clearly separate effects of xylene from those of other exposures, and had few subjects exposed (Sallmén et al., 1998) or were not reported in detail (Xiao et al., 1999, 2001). Of the four animal studies, only the Biodynamics (1983) study is of a quality appropriate for quantitative risk assessment. The other available animal studies provide some useful information, but must be considered in light of limitations such as small or unstated group size (Nylen et al., 1983; Washington et al., 1983; Yamada, 1993), route of exposure not experienced by humans (Washington et al., 1983), lack of information on systemic toxicity (Nylen et al., 1983; Washington et al., 1983; Yamada, 1993), and lack of information on the test concentration (Yamada, 1993).

D. Female Reproductive Toxicology Studies

D.1. Human Female Reproductive Studies

Two studies examined female reproductive endpoints in relation to maternal occupational exposure to xylene and other organic solvents. These studies are summarized in Section D.1.1 below, in Table 4, and in greater detail in Appendix A.

Several studies examined human female reproductive outcomes in relation to more general exposures, such as laboratory work or work with organic solvents, but did not report associations specifically for xylene. These studies also examined fecundability (Wennborg et al., 2001) and menstrual cycle function (Reutman et al., 2002), as well as luteal function (Chen et al., 2000) and pregnancy syndromes or reactions (Wang, 1994, Yang, 1997). Brief summaries of these studies are presented in Section D.1.2.

Epidemiologic studies that examine outcomes such as SAB are discussed in the developmental toxicity section of this document. Outcomes such as SAB may be manifestations of direct toxicity to the conceptus, but may also be mediated in part or in whole through toxicity to the reproductive system of the mother. That is, the effect may be directly on the conceptus, on the female reproductive system, or both. Mechanistic data can clarify this, but such data are not available for xylene. Thus, the studies reviewed in the developmental toxicity sections E.1.1 and E.1.2 can also be considered in the context of identifying female reproductive toxicity.

D.1.1. Human Female Reproductive Studies of Xylene

To study the association between time to pregnancy (TTP) and solvent exposure, Sallmén et al. (1995) constructed a cohort of subjects from the cases and controls of earlier studies of SAB (Lindbohm et al., 1990) and congenital malformations (unpublished). Information about TTP, contraceptive use, menstrual cycles, lifestyle, and other potentially related factors was collected from 197 subjects by mailed questionnaires. Occupational exposure information had been collected for the previous studies. If a woman reported in the TTP questionnaire that her work tasks had changed during the TTP, her exposure was re-assessed. Sallmén et al. used discrete proportional hazards regression with the ~10-day periovulatory period within menstrual cycles representing the disjointed time intervals in the background to estimate the incidence density ratios (IDR) of clinically recognized pregnancies for exposed vs. unexposed women. An IDR < 1 indicates decreased fertility. The IDRs for xylene were 1.41 (CI 0.91-2.20) for low exposure, and 0.93 (CI 0.47-1.84) for high exposure, adjusted for exposure to other solvents, recent contraceptive use, and age at menarche.

Cho et al. (2001) conducted a cross-sectional study in a petrochemical industry in Beijing, China to examine whether there is an association between low-level exposure to organic solvents and menstrual patterns. The major occupational exposures included aromatic solvents such as benzene, toluene, styrene, and xylene. The subjects were

20-40 year old married women who had never had children. The final sample size was 1,408 and the participation rate was 95% of eligible women. Exposure assessment was based on classification of workshops as either exposed or unexposed to each solvent and women's reports of handling specific chemicals. No women were exposed to xylene alone. Among women exposed to xylene (with other solvents), 14.1% had oligomenorrhea (defined as an average cycle length greater than 35 days). The OR (CI) for oligomenorrhea and exposure to xylene (with other solvents) was 1.63 (1.04-2.53), adjusted for age, body mass index, enrollment cohort, passive smoking, and exposure to chemicals other than aromatic solvents. "Any aromatic solvent" exposure was associated with a 34% increase in the odds of oligomenorrhea. The OR (CI) for exposure to "all aromatic solvents" was 1.76 (1.08-2.82) compared to the unexposed group. The OR (CI) for oligomenorrhea and exposure to any aromatic solvent for more than 3 years, compared with no exposure, was 1.53 (1.00-2.34).

Table 4. Human Female Reproductive Studies

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Sallmén et al., 1995 Finland Births 1973-1983 Objective: to investigate whether maternal occupational exposure to organic solvents is associated with reduced fertility, as indicated by time-to-pregnancy (TTP)	Retrospective cohort 197 women who were biomonitored from 1965-1983 for xylene or 5 other solvents. Group was drawn from previous case-control studies of SAB and malformations. Data on TTP and related factors were collected by mailed questionnaire. Discrete proportional hazards regression, with the ~10-day periovulatory period within menstrual cycles representing the disjointed time intervals in the background. The outcome is an estimate of the ratio of average incidence densities of clinically recognized pregnancies for exposed vs. unexposed women.	Data on employment, workplace, and occupational exposure during the 1st trimester were obtained by mailed questionnaire. Subjects were asked to describe work tasks in detail, including specific tasks involving solvents, handling of the 6 monitored solvents, other solvents, and frequency of handling. Although all of the women were biomonitored because they worked with organic solvents, few of the measurements were relevant to the TTP study. Authors classified likelihood and level of exposure, based on reported occupation, work description, reported use of solvents during the pre-pregnancy period, and biological exposure measurements when available.	Factors related to conception, including: planned pregnancy, frequency of intercourse, menstrual cycles without contraception before getting pregnant, breast feeding, stress, lifestyle factors (e.g., smoking, alcohol, caffeine consumption), partner's smoking and history of illness or other relevant condition, heavy lifting, pregnancy and work histories, handling of other solvents, indicator variable for SAB case.	Adjusted incidence density ratios for women exposed to xylene (compared to not exposed): Low exposure: 1.41 (CI 0.91-2.20; n=31); High exposure: 0.93 (CI 0.47-1.84; n=10), adjusted for other solvents, recent contraceptive use, age at menarche. Exposure to organic solvents was associated with longer TTP and reduced fecundability.	Measured levels of xylene were low, with no levels exceeding the Finnish Threshold Limit Value. TTP data were collected 8-18 years after the pregnancies. The participation rate in this study was relatively low (66%). Most women exposed to aromatic hydrocarbons were also exposed to other solvents.

Table 4. Human Female Reproductive Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Cho et al., 2001 Beijing, China 1994-1998 Objective: to examine association between low-level exposure to organic solvents and menstrual patterns in women employed in a petrochemical industry	Cross-sectional 1,408 non-parous, married, 20-40 year old women who worked in petroleum and chemical processing plants were identified through district health centers or family planning offices. The participation rate was 95%. Menstrual patterns were assessed in a baseline interview. Oligomenorrhea was defined as average cycle length > 35 days, polymenorrhea as average cycle length < 21 days, and menorrhagia as average bleeding period > 7 days. Multiple logistic regression	Based on previous research, each workshop was classified for exposure to benzene, toluene, styrene, and/or xylene. Women also reported handling of specific chemicals on a checklist in the baseline questionnaire.	The authors collected detailed information on age, weight, height, date of marriage, current and past contraceptive use, parity, history of active and passive smoking, indoor coal combustion and cooking oil fumes, alcohol use, diet, use of herbal medicines, heavy lifting, body position during work, rotating shift work, perceived work stress, and physical activities outside the workplace.	No women were exposed to xylene alone. Among women exposed to xylene (with other solvents), 14.1% had oligomenorrhea. **The OR for exposure to xylene and oligomenorrhea was 1.63 (CI 1.04-2.53), adjusted for age, body mass index, enrollment cohort, passive smoking, and exposure to chemicals other than aromatic solvents.** The adjusted OR for oligomenorrhea and exposure to any aromatic solvent was 1.34 (0.90-1.99) The adjusted OR for each year of exposure to any aromatic solvent was 1.07 (1.00-1.14). For exposure to any aromatic solvent for > 3 years, compared with no exposure, the adjusted OR was 1.53 (1.00-2.34).	The authors report that because the industry is modern, exposures are very low, with average levels of benzene well below the limit recommended by the National Institute for Occupational Safety and Health, and xylene, toluene, and styrene levels below 1 ppm.

D.1.2. Related human female reproductive studies

Several studies examined female reproductive outcomes in relation to organic solvents, but did not report associations specifically for xylene.

Wennborg et al. (2001) conducted a retrospective cohort study to identify potential factors in the laboratory environment that could be associated with reduced fecundability. The cohort included women who had worked in university biomedical research laboratories (exposed) or certain non-laboratory departments (unexposed). TTP was used to estimate fecundability, and the measure of effect was the fecundability ratio (FR) between exposed and unexposed cycles. The FR for occupational exposure to solvents, adjusted for cycle order, mother's age, father's age, father's laboratory work, and reported fertility problems, was 0.79 (0.68-0.93).

Reutman et al. (2002) conducted a cross-sectional study to assess the potential effects of fuel and solvent exposure (including benzene, toluene, ethylbenzene, and xylenes [BTEX]) on menstrual cycle function in female U.S. Air Force civilian and active military employees. Participants (n=170) were administered a baseline questionnaire on work, reproductive and menstrual history, and potentially related factors, and provided first morning urine samples for endocrine data. The key endocrine end points, chosen *a priori* based on association with non-conception during ovulatory cycles, were:

- elevated levels of follicular pregnanediol 3-glucuronide (Pd3G),
- reduced levels of preovulatory luteinizing hormone (LH),
- midluteal Pd3G, and
- midluteal estrone 3-glucuronide ($E_1$3G).

Internal doses of BTEX were estimated from mixed-exhaled breath samples from 63 participants. High breath BTEX levels were associated with lower mean preovulatory LH (p=0.03) and midluteal Pd3G (p=0.06). When analyzed as a continuous variable, total BTEX was not significantly associated with any of the measured hormone levels.

Chen et al. (2000) investigated effects of occupational exposure to BTX on luteal function. The study sample comprised 50 exposed women who worked at an oil refinery company, 35 internal controls who worked at and lived near the same company but were not directly exposed at the production lines, and another 35 general controls who worked at a chemical fiber company. Eligibility criteria included aspects of contraceptive and reproductive history. Benzene was detected in 29.1% of air samples, toluene was detected in 7.6% of air samples, and xylene was detected in 2.7% of all air samples. Most detected levels of benzene and its analogues were well below the state limit. The mean luteal phase was significantly shorter among exposed women than in either control group. Short luteal phase (≤ 10 days) was more common among the exposed than among either control group (18.0% vs. 2.9% and 2.9%; p=0.04 for both comparisons). Urinary concentrations of Pd3G during the early follicular phase and luteal phase were also lower in the exposed group. The proportion of workers with luteal malfunctions (defined as a Pd3G level increased by at least three-fold but less

than six-fold) was higher among exposed women than controls (28.0% vs. 8.6%; p=0.05).

Wang (1994) examined pregnancy syndromes, menstrual cycles, and pregnancy outcomes among 161 women who were exposed to benzene and its analogues through their work in the painting industry, compared with 154 unexposed women who worked in hotels and were matched to exposed women by age and other characteristics. Exposed women were significantly more likely to have abnormal menstrual cycles, heavy bleeding, and cramps. Pregnancy syndromes of severe reactions (not defined), anemia, and hypertension were also more common among exposed women. A larger proportion of pregnancies ended in abortion among exposed women.

Yang (1997) reported on a survey of occupational diseases among female workers in Liaoning Province, China, conducted by the provincial government. The study population included 3,248 women exposed to benzene and its analogues (BIA) through work in the rubber, leather, chemical, painting and insulation industries. The unexposed were 7,247 women working in the postal service, retail, and services. The two groups were similar in terms of marital status, age, education, years of work, passive smoking and other measures of socioeconomic status. Significantly more exposed women than unexposed women had irregular menstrual cycles (39.2% vs. 15.0%; p<0.005) and severe menstrual pain (3.1% vs. 0.5%; p<0.005). The following pregnancy complications were also more prevalent among exposed than unexposed women: severe reaction[3] (12.3% vs. 8.5%; p<0.005), abortion signs (0.6% vs. 0.09%; p<0.005), abortion (6.2% vs. 3.3%; p<0.005), anemia (6.4% vs. 5.3%; p<0.005), preterm birth (1.6% vs. 0.5%), and delayed birth (0.4% vs. 0.03%). The offspring of exposed women were also more likely to experience the following problems: low birth weight (8.4% vs. 3.0%; p<0.005), mental retardation (7.1% vs. 0.6%; p<0.005), and newborn death (5.7% vs. 2.0%; p<0.005).

D.2. Female Animal Reproductive Toxicology Studies

Only one reproductive toxicity study of xylene was identified: a one-generation inhalation reproductive toxicity study performed in rats (Biodynamics, 1983). Hence, this is the only study to evaluate endpoints of female reproductive toxicity such as mating index and fertility. There are no studies evaluating effects of xylene on the estrous cycle.

Additional information relevant to the female reproductive toxicity of xylene is available from studies of developmental toxicity that include maternal and litter data. In these studies, xylene exposure was limited to some part of gestation.

Eleven developmental toxicity studies which provided evidence about female reproductive toxicity were conducted by the inhalation route of exposure (Hass and Jakobsen, 1993; Hass et al., 1995; Hass et al., 1997; Hudak and Ungvary, 1978; Litton

[3] "Severe reaction" was not defined in the report, but is interpreted by OEHHA as a commonly used clinical term for severe morning sickness, i.e., nausea and vomiting.

Bionetics, 1978; Mirkova et al., 1983; Rosen et al., 1986; Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary et al., 1981; Ungvary and Tatrai, 1985). One developmental toxicity study conducted by the oral route of exposure provided information about female reproductive toxicity (Marks et al., 1982).

D.2.1. Information on Female Reproductive Toxicity from Reproductive Toxicity Studies

In a large one-generation reproductive toxicity study, male and female CD rats were exposed to mixed xylenes by inhalation for 131 days pre-mating, and 20 days of mating (Biodynamics, 1983). Pregnant females continued on their treatment regimen throughout gestation and lactation, except for the days between gestation day (GD) 20 and PND 5. Lactating females were separated from their litters during the treatment periods.

Animals were exposed daily, for six hours per day, to xylene concentrations of 0, 60, 250, or 500 ppm. Two groups of females were exposed to the high concentration of 500 ppm xylene. In one of these groups, both males and females were treated. In the other group, only females were exposed.

No clinical symptoms of toxicity were reported for the treatment period. No mortality occurred among treated animals. Maternal body weight was not affected by xylene treatment, and no adverse effects were observed at gross postmortem evaluation. One of two groups of females exposed to 500 ppm xylene showed an increase in mean kidney weight, and an increased mean corrected body weight at GD 21. Mean kidney to body weight ratio for these females showed a nonsignificant increase as compared to controls.

Pregnancy and fertility indices did not differ among groups. Compared to controls, lower mating indices were observed among the females exposed to 250 ppm xylene and females exposed to 500 ppm xylene and paired with untreated males. Mating indices were not affected in any of the other groups. Observed variation among groups in time-to-mating were not considered to result from treatment.

Xylene was not shown to affect gestation length or parturition. The females sacrificed before term for the teratology part of the study (0 or 500 ppm xylene) showed no differences between groups for numbers of corpora lutea, implantation frequency, resorption frequency, or numbers of live fetuses.

Among the females allowed to deliver their litters normally, a significant increase in live litter size ($P < 0.05$) was seen at 250 ppm xylene, and in one group exposed to 500 ppm xylene. These differences seem likely related to the small mean litter size among controls: 9.6 live pups per litter on PND 1 for controls, as compared to 10.8-12.5 live pups per litter among treated groups. No significant difference from controls in litter size was seen for females exposed to 60 ppm xylene, or those in the second group of females exposed to 500 ppm xylene.

Ovary weights for F1 pups taken on PND 21 and 49 gave no evidence of an effect of xylene exposure.

D.2.2. Information on Female Reproductive Toxicity from Developmental Toxicity Studies by the Inhalation Route of Exposure

Additional information on the female reproductive toxicity of xylene by the inhalation route of exposure comes from developmental toxicity studies in which exposure was limited to the gestation period (Hass and Jakobsen, 1993; Hass et al., 1995; Hass et al., 1997; Hudak and Ungvary, 1978; Litton Bionetics, 1978; Mirkova et al., 1983; Rosen et al., 1986; Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary et al., 1981; Ungvary and Tatrai, 1985). As with epidemiologic studies, some developmental outcomes in animals may be manifestations of direct toxicity to the conceptus, but may also be mediated in part or in whole through toxicity to the reproductive system of the mother. That is, the effect may be directly on the conceptus, on the female reproductive system, or both.

Hass and Jakobsen (1993) exposed Wistar rats to 200 ppm xylene for six hours per day on GD 6-20. Some animals were sacrificed on gestation day 21 and their uterine contents evaluated; other females were allowed to deliver their litters normally.

No clinical signs of maternal toxicity were observed. Maternal body weight, food consumption, and uterine weight on gestation day 21 were not affected by treatment. No effects of xylene exposure were observed on the numbers of corpora lutea, implantation frequency, or the numbers of live, dead, or resorbed fetuses. Preimplantation loss was slightly increased in treated (10.2%), relative to control (6.0%), litters, but this difference was not statistically significant. For the litters delivered normally, no differences were seen between treated and control groups in gestation length, number of pups per litter, or sex ratio.

In two additional inhalation studies of pregnant Wistar rats, Hass et al. (1995 and 1997) exposed animals to 0 or 500 ppm xylene for six hours per day on GD 7-20. Dams were allowed to deliver their litters normally. No evidence was found for maternal systemic toxicity or female reproductive toxicity. Xylene exposure had no effect on maternal gestational weight gain or gestation length. Implantation frequency and the numbers of live pups per litter were also not affected by xylene exposure.

Hudak and Ungvary (1978) exposed pregnant rats to 0 or 230 ppm xylene for 24 hours per day, from GD 9-14. No maternal mortality occurred, and no effects of xylene were observed on maternal gestational weight gain. Treatment had no significant effect on fetal loss or mean litter size.

Litton Bionetics (1978) exposed pregnant rats to xylene by inhalation at concentrations of 0, 100, or 400 ppm, six hours per day on each of GD 6-15. Maternal body weights and feed consumption did not vary among groups. No gross visceral pathology was noted at necropsy. There were no differences among groups in litter variables including: frequencies of live litters, resorption frequency, or mean live litter size.

Mirkova et al. (1983) exposed pregnant Wistar rats to xylene by inhalation at concentrations of 0, 10, 50, or 500 mg/m^3 (0, 2.31, 11.53, or 115.33 ppm), for 6 hours per day, five days per week, from GD 1-21. On GD 21, some of the animals were sacrificed for fetal evaluation, while others were allowed to deliver their litters normally.

The paper includes no discussion of maternal weights, or other evidence of maternal toxicity. Most of the common litter variables showed no significant effect of treatment. Unchanged variables included: gestation index, mean number of corpora lutea per litter, mean implantations per litter, mean live fetuses per litter, and resorptions as a percent of total implantations. On the other hand, the percentages of post implantation loss and autolyzed fetuses per total implantations were significantly increased at the two highest concentrations of xylene ($p < 0.05$ and $p < 0.01$, respectively). It is difficult to interpret the meaning of these last two parameters, considering the lack of effect on live litter size and implantation frequency.

Rosen et al. (1986) exposed groups of 25 pregnant Sprague-Dawley rats to 0, 3500, or 7000 mg/m^3 (0, 807.31, or 1614.62 ppm) p-xylene on GD 7-16. Dams gave birth normally, at which time their pups were counted and weighed. A significant reduction was reported for maternal weight gain during treatment in the high-dose group, although data and details were not provided. Xylene exposure had no effect on the number of females giving birth in each group or on litter size.

Saillenfait et al. (2003) exposed Sprague-Dawley rats to ethylbenzene, o-, m-, p-xylene, or technical xylene by the inhalation route of exposure. Each agent was provided at concentrations of 0, 100, 500, 1000, or 2000 ppm. The duration of exposure was six hrs/day, on each of GD 6-20.

Each of the agents tested resulted in reduced gestational weight gain at 1000 and 2000 ppm. Decreases in corrected maternal weight gain (accounting for the pregnant uterus) and feed consumption were observed with 2000 ppm technical xylene, as well as with both 1000 and 2000 ppm of the other test compounds. None of the test compounds, at any concentration, showed significant effects on maternal mortality, pregnancy rate, corpora lutea, implantation sites, percentages of live and dead fetuses per litter, resorption frequency, or fetal sex ratio.

Ungvary et al. (1980) exposed pregnant CFY rats by inhalation to individual xylene isomers at concentrations of 0, 150, 1500, or 3000 mg/m^3 (0, 35, 346, or 692 ppm). Animals were kept in the exposure chambers for 24 hours per day on GD 7-14.

Death occurred in 4/30 females exposed to the highest concentration of m-xylene, but no maternal mortality was seen in any of the other groups. The numbers of non-pregnant animals at term varied among groups and did not seem to show a dose-related pattern.

Gestational weight gain decreased with increasing dose for each xylene isomer, but only reached statistical significance at the high concentration of m-xylene ($P < 0.05$). Maternal food consumption was decreased during the treatment period with exposure to the high concentration of o-xylene, and the medium and high concentrations of m- and p-xylene. Feed consumption returned to normal, or exceeded control levels following the end of the treatment period. The liver/body weight ratio was significantly lower than controls ($P < 0.05$) for all concentrations of o-xylene, but did not show a clear dose effect. Liver/body weight ratio was not affected in any of the other groups.

Seven out of 20 litters in the high concentration group of p-xylene were totally resorbed, as were 2/20 litters in the high concentration group of o-xylene. Mean litter size and fetal loss expressed as a percentage of implantation sites were altered only at the highest concentration of p-xylene. A significant decrease in the mean number of implantation sites per dam was seen only at the high concentration of m-xylene ($p < 0.05$).

Ungvary et al. (1981) exposed CFY rats to 0 or 3000 mg/m^3 (0, 692 ppm) p-xylene on GD 10 or GD 9-10. Exposure was continuous either for 24 hours, or for 48 hours. There were 8 to 14 pregnant females in each group. Uterine and ovarian venous blood flow, ovarian progesterone and 17ß-estradiol secretion, and levels of progesterone and 17ß-estradiol in peripheral blood were measured on GD 11.

No significant effects were seen on blood flow in the ovary or uterus with exposure to p-xylene. Nor were they are any significant effects of p-xylene on ovarian progesterone or 17ß-estradiol secretion. Peripheral blood levels of progesterone and 17ß-estradiol secretion were not affected by 24 hours of exposure to p-xylene, but both hormones were significantly decreased ($p < 0.05$) by 48 hours of exposure.

Ungvary and Tatrai (1985) studied the effects of technical xylene and/or its individual isomers by inhalation in pregnant rats, mice, and rabbits. Exposure protocols were as follows:

- CFY rats
  - Technical xylene at concentrations of 0, 250, 1900, or 3400 mg/m^3 (0, 58, 438, or 784 ppm)
  - 24 hrs/day on GD 7-15; N = 20-23
- CFLP mice
  - Technical xylene or one of its individual isomers at concentrations of 0, 500, or 1000-1500 mg/m^3 (0, 115, or 231-346 ppm)
  - Either 24 hrs/day, or for three sessions of four hours each per day, on GD 6-15; N = 15-18 (115 controls)
- NZ rabbits
  - Technical xylene or one of its isomers at concentrations of 0, 500, or 1000 mg/m^3 (0, 115, or 231 ppm)
  - 24 hours/day on gestation days 7-20; N = 9-10 (60 controls)

Results for each species were as follows:

- Rats
  - 1/20 high-concentration group dams died
  - No effect on the numbers of live fetuses per group
  - Increased percentage of dead or resorbed fetuses at the high concentration of 784 ppm ($p \leq 0.05$)
- Mice
  - No effect of xylene or any of its isomers on maternal mortality
  - No effect of xylene or any of its isomers on the numbers of live fetuses, or the percentage of dead or resorbed fetuses
- Rabbits
  - No effect of xylene or any of its isomers on maternal mortality or percent gestational weight gain
  - Significant increase in percent relative maternal liver weight for the high concentration of technical xylene only ($p \leq 0.05$)
  - 1/8 and 3/10 dams died at 231 ppm p-xylene and technical xylene, respectively
  - 3/8 and 6/10 litters were aborted at 231 ppm p-xylene and technical xylene, respectively
  - 1/10, 4/8, and 1/10 litters were totally resorbed or dead at 115 ppm p-xylene, 231 ppm p-xylene, and 231 ppm technical xylene, respectively
  - The percentage of dead or resorbed fetuses, among total fetuses, was significantly increased at 115 ppm m-xylene ($P \leq 0.05$)

D.2.3. Information on Female Reproductive Toxicity from Developmental Toxicity Studies by the Oral Route of Exposure

Marks et al. (1982) gave xylene to pregnant CD-1 mice by oral gavage in a cottonseed oil vehicle. Treatments were given three times per day, on each of GD 6-15. The doses used were 0, 0.52, 1.03, 2.06, 2.58, 3.10, and 4.13 mg/kg-day.

None of the 15 pregnant dams exposed to the highest dose of 4.13 mg/kg-day survived until gestation day 18. Maternal mortality was 12 of 38 treated dams in the 3.10 mg/kg-day dose group. All the pregnant females in all of the other dose groups survived the study.

Other maternal endpoints included:

- Significantly decreased maternal gestational weight gain ($p < 0.05$) at the highest survivable dose of 3.10 mg/kg-day
- Significantly increased maternal liver weights compared to controls at doses of 2.06 and 2.58 mg/kg-day ($p < 0.05$, at both doses)
- Mean gravid uterine weights were significantly decreased compared to controls at doses of 2.06, 2.58, and 3.10 mg/kg-day ($p < 0.05$, for each dose), in a dose-dependent manner

Litter endpoints included:

- No effect on implantation rate, sex ratio, or mean live litter size
- At the highest tolerated dose of 3.10 mg/kg-day, 13/20 litters were completely resorbed
- The frequency of resorptions out of total implants was 62.3% at the highest tolerated dose of 3.10 mg/kg-day ($p < 0.05$)

Table 5. Animal Studies of Female Reproductive Toxicity

Reference	Study design	Systemic toxicity	Female reproductive toxicity
Biodynamics (1983)	1-generation reproduction study; CD rats; N=10-30; 0, 60, 250, and 500 ppm; inhalation; daily, 6 hr/day; 131-days pre-mating, 20 day mating period, gestation and lactation	No mortality in treated animals No clinical symptoms of toxicity No effect on body weight No gross pathology	No effects on pregnancy or fertility indices ↓ Mating indices at 250 ppm and one group at 500 ppm (only ♀ treated) No effect at 500 ppm, both ♀ and ♂ treated Variation in time-to-mating not considered due to treatment
Hass and Jakobsen (1993)	Developmental toxicity study; Wistar rats; N= 36; 0, 200 ppm xylene; inhalation; 6 hrs/day,GDs 4-20 2/3 ♀ sacrificed on GD 21, remainder delivered normally	No clinical of toxicity No effect on body weight, food consumption, or uterine weight on GD-21	No effects on the numbers of corpora lutea, implantation frequency, or the numbers of live, dead, or resorbed fetuses. Preimplantation loss slightly ↑ in treated animals (10.2%) relative to control (6.0%), not statistically significant. For litters delivered normally, no differences between groups in gestation length, number of pups per litter, or sex ratio
Hass et al. (1995)	Developmental toxicity, Mol:WIST rats; inhalation; 0, 500 ppm xylene; N = 13-15 6 hrs/day on GDs 7-20. Dams allowed to deliver normally	No effect on maternal gestational weight gain	No effect on gestation length No significant effects on implantations per litter or the numbers of live pups per litter
Hass et al. (1997)	Developmental toxicity, Mol:WIST rats; inhalation; 0, 500 ppm xylene; N = 13-15 6 hrs/day on GDs 7-20. Dams allowed to deliver normally	No evidence for maternal toxicity	No evidence of embryo-and fetotoxicity

Table 5. Animal Studies of Female Reproductive Toxicity (continued)

Hudak and Ungvary (1978)	Developmental toxicity, CFY rats; inhalation; 0 or 1000 mg/m^3 (230 ppm) xylene; N = 20-28; 24 hrs/day, GD 9-14	No effect on maternal mortality or maternal gestational weight gain	No effects on fetal loss or mean litter size
Litton Bionetics (1978)	Developmental toxicity , Rat; inhalation; 0, 100, 400 ppm xylene; 6 hr/day, GD 6-15; N = 26	No effect on maternal mortality, gestational weight gain, or food consumption; no evidence of organ pathology	No significant effect on mean live litter size, live litters/bred ♀s, litters with resorptions, litters with dead fetuses, or live fetuses/implantation sites
Mirkova et al. (1983)	Developmental toxicity; Wistar rats; inhalation; 0, 10, 50, or 500 mg/m^3 (0, 2.31, 11.53, or 115.33 ppm); 6 hrs/day, 5 days/ week, GD 1-21	No mention of maternal mortality or weight gain	No change in gestation index, corpora lutea/ litter, implantations/ litter, live fetuses/ litter, or resorptions as % of implantations ↑ post implantation loss and autolyzed fetuses/ implantations at 11.53 and 115.33 ppm
Rosen et al. (1986)	Developmental toxicity; Sprague-Dawley rats; inhalation 0, 3500, 7000 mg/m^3 (0, 807.31, 1614.62 ppm) p-xylene; GD 7-16; N = 25 Dams gave birth normally	↓ maternal weight gain during treatment in the high-dose group	No effect on number of females giving birth /group or litter size
Saillenfait et al. (2003)	Developmental toxicity; Sprague-Dawley rats; inhalation ethylbenzene, o-, m-, p-xylene, or technical xylene 0, 100, 500, 1000, or 2000 ppm; 6 hrs/day, GD 6-20; N = 20-26	No effect on maternal mortality ↓ gestational wt gain, 1000 and 2000 ppm, all compounds ↓ corrected maternal wt gain and feed consumption, 2000 ppm technical xylene, and 1000 and 2000 ppm of other compounds	No effect on pregnancy rate, number of corpora lutea, number of implantation sites, percentages of live and dead fetuses per litter, resorption frequency, or fetal sex ratio

Table 5. Animal Studies of Female Reproductive Toxicity (continued)

Ungvary et al. (1980)	Developmental toxicity; CFY rats; inhalation, individual xylene isomers: 0, 150, 1500, or 3000 mg/m^3 (0, 35, 346, or 692 ppm); 24 hr/day, GD 7-14; N = 15-30	Maternal mortality 4/30 ♀ at 692 ppm m-xylene ↓ Gestational wt gain at 692 ppm m-xylene ↓ Liver/body wt ratio at all concentrations of o-xylene, no clear dose effect	Total resorption of 7/20 litters at 692 ppm p-xylene and 2/20 litters at 692 ppm o-xylene ↓ Mean litter size and fetal loss/implantation sites at 692 ppm p-xylene ↓ Implantation sites/dam at 692 ppm m-xylene
Ungvary et al. (1981)	CFY rats; inhalation, p-xylene: 0, 3000 mg/m^3 (0, 692 ppm); GD 10 or GD 10 (continuous 24 or 48 hrs); N = 8-14	No information provided	No effects on ovarian or uterine blood flow No effects on ovarian progesterone or 17ß-estradiol secretion No effect of 24 hrs exposure, but peripheral blood levels of progesterone or 17ß-estradiol ↓ by 48 hrs exposure
Ungvary and Tatrai (1985)	Developmental toxicity; inhalation CFY rats; 0, 250, 1900, or 3400 mg/m^3 (0, 58, 438, or 784 ppm) ; 24 hrs/day, GD 7-15; N = 20-23 CFLP mice; xylene or single isomers; 0, 500, or 1000-1500 mg/m^3 (0, 115, or 231-346 ppm); 24 hrs/day or 3, 4-hr sessions/day, GD 6-15; N = 15-18 (115 controls) NZ rabbits; xylene or single isomers; 0, 500, or 1000 mg/m^3 (0, 115, or 231 ppm); 24 hrs/day, GD 7-20; N = 9-10 (60 controls)	Rats: 1/20 dams died at 784 ppm xylene Mice: no effects on maternal mortality Rabbits: 1/8 and 3/10 dams died at 231 ppm p-xylene and technical xylene, respectively; ↑ % maternal liver weight at 231 ppm technical xylene	Rats: ↑ % dead or resorbed fetuses at 784 ppm Mice: no effects reported Rabbits: 3/8 and 6/10 litters aborted at 231 ppm p-xylene and technical xylene, respectively; 1/10, 4/8, and 1/10 litters totally resorbed or dead at 115 ppm p-xylene, 231 ppm p-xylene, and 231 ppm technical xylene, respectively; ↑ % dead or resorbed fetuses at 115 ppm m-xylene

Table 5. Animal Studies of Female Reproductive Toxicity (continued)

Marks et al. (1982)	Developmental toxicity; CD-1 mice; oral gavage; 0, 0.52, 1.03, 2.06, 2.58, 3.10, and 4.13 mg/kg-day; dosing 3/day, GD 6-15; N= 23-66	Total lethality at high dose of 4.13 mg/kg-day 32% mortality at 3.10 mg/kg-day All other dams survived ↓ Gestational wt gain at 3.10 mg/kg-day ↑ Liver wts at 2.06 and 2.58 mg/kg-day	↓ gravid uterine wts, dose-dependent, 2.06, 2.58, and 3.10 mg/kg-day No effect on implantation rate, sex ratio, or mean live litter size ↑ resorptions at 3.10 mg/kg-day

D.3. Integrative Evaluation for Female Reproductive Toxicity

Two epidemiologic studies examined effects of occupational exposure to xylene on female reproductive outcomes, specifically TTP and menstrual patterns, and developmental studies examined SAB. Only one reproductive toxicity study of xylene in animals was identified: a one-generation inhalation reproductive toxicity study performed in rats (Biodynamics, 1983). This was the only animal study to evaluate endpoints of female reproductive toxicity such as mating index and fertility. There are no animal studies evaluating effects of xylene on the estrous cycle, although one study did consider secretion and circulation of ovarian progesterone and 17ß-estradiol following xylene exposure of pregnant rats (Ungvary et al., 1981).

Additional information relevant to the female reproductive toxicity of xylene in animals is available from studies of developmental toxicity in which xylene exposure was limited to part or all of gestation. Twelve animal developmental toxicity studies included data on endpoints such as numbers of corpora lutea, implantation frequency, resorption frequency, and numbers of live and dead fetuses or newborns (Hass and Jakobsen, 1993; Hass et al., 1995; Hass et al., 1997; Hudak and Ungvary, 1978; Litton Bionetics, 1978; Marks et al., 1982; Mirkova et al., 1983; Rosen et al., 1986; Saillenfait, et al., 2003; Ungvary et al., 1980; Ungvary et al., 1981; Ungvary and Tatrai, 1985). Eleven developmental toxicity studies were performed by the inhalation route of exposure in rats, with one study using mice and rabbits in addition to rats. The single oral developmental toxicity study was performed in mice.

Out of the 13 animal studies providing information relevant to evaluating female reproductive toxicity, only three showed any evidence of xylene-induced female reproductive toxicity. The strongest effects reported were embryo-fetal mortality observed in rabbits, at inhalation concentrations that were also associated with maternal mortality (Ungvary and Tatrai, 1985). P-xylene appeared to be the most effective isomer, but technical and m-xylene were also associated with embryo-fetal death. As in rabbits, some evidence for a particular effect of p-xylene was observed in rats (Ungvary et al., 1980), but effects were also seen with inhalation exposure to o- and m-xylene. In this study, only m-xylene was associated with maternal mortality. Litter effects providing

evidence for reproductive toxicity consisted of totally resorbed litters, reduced mean litter size, and reduced mean number of implantation sites. Oral exposure of mice to xylene was also associated with maternal death at high doses, and with increased resorptions at the highest tolerated dose (Marks et al., 1982).

Epidemiologic studies examining SAB, discussed in the context of developmental effects in Section E.1., provide mixed evidence for exposure to xylene as a risk factor for fetal loss. Two studies found significantly increased risk of SAB among female laboratory workers (Taskinen et al., 1994) and women working in the semiconductor industry (Swan et al., 1995), while three other studies reported either non-significant associations (Lindbohm et al., 1990 and Windham et al., 1991), or no association (Axelsson et al., 1984) between maternal xylene exposure and risk of SAB.

The epidemiologic study of TTP found TTP was not significantly different in women occupationally exposed to xylene (Sallmén et al., 1995). In animals, exposure to xylene was not associated with effects on pregnancy or fertility indices, or on time-to-mating (Biodynamics, 1983). Nor were effects observed in animal studies on numbers of corpora lutea (Hass and Jakobsen, 1993; Mirkova et al., 1983; Saillenfait, et al., 2003) or implantation frequency (Hass et al., 1995; Hass and Jakobsen, 1993; Mirkova et al., 1983; Saillenfait, et al., 2003; Marks et al., 1982).

The only epidemiologic study that examined effects of occupational exposure to xylene on menstrual cycles found that low-level exposure to xylene was associated with oligomenorrhea in women working in petroleum and chemical processing plants in China (Cho et al., 2001), though the women were exposed to multiple other industrial chemicals that could have contributed to the observed association. No animal studies evaluated estrous cycles in females exposed to xylene.

One study, Ungvary et al. (1981), used CFY rats to look at uterine and ovarian venous blood flow, ovarian progesterone and 17ß-estradiol secretion, and levels of progesterone and 17ß-estradiol in peripheral blood . Exposed animals were evaluated at 24 hours following either 24 or 48 hours of continuous xylene exposure. No significant effects were seen on blood flow in the ovary or uterus with exposure to p-xylene. Nor were they are any significant effects of p-xylene on ovarian progesterone or 17ß-estradiol secretion. Peripheral blood levels of progesterone and 17ß-estradiol secretion were not affected by 24 hours of exposure to p-xylene, but both hormones were significantly decreased by 48 hours of exposure.

E. Developmental Toxicity Studies

E.1. Human Developmental Toxicity Studies

Nine epidemiologic studies examined associations between developmental outcomes and xylene (Axelsson et al., 1984; Ghosh et al., 2012; Lehman et al., 2002; Lindbohm et al., 1990; Lupo et al., 2011; Swan et al., 1995; Taskinen et al., 1989; Taskinen et al., 1994; Windham et al., 1991). Developmental outcomes studied include congenital malformations, SAB, fetal growth (e.g., low birth weight [LBW]), and one study that examined T cell cytokines. These studies are briefly described below and in Table 6. More detailed summaries of the studies are in Appendix A. As with other endpoints, most studies of developmental endpoints reported results for xylene but could not separate potential effects of xylene from those of other organic solvents. Studies that examined exposure to xylene and/or organic solvents without reporting results for xylene follow in Section E.1.2, under the heading "Related Human Developmental Studies."

E.1.1. Human Developmental Toxicity Studies of Xylene

Lupo et al. (2011) conducted a case-control study to assess the association between maternal exposure to environmental levels of benzene, toluene, ethylbenzene, and xylene (BTEX) and neural tube defects (NTDs; spina bifida and anencephaly) in offspring. Benzene was the primary pollutant of interest. Data on live births, stillbirths, and electively terminated fetuses with NTDs delivered from 1999-2004 were obtained from the Texas Birth Defects Registry. Controls were a stratified random sample of unaffected live births, selected at a ratio of four controls per case and frequency matched to cases by year. Analyses included 533 spina bifida cases, 303 anencephaly cases, and 3,695 controls. Estimates of annual ambient BTEX concentrations for each mother's census tract were obtained from the U.S. EPA 1999 Assessment System for Population Exposure Nationwide (ASPEN), which uses emissions data, meteorological conditions, and other information. Mixed-effects logistic regression was used to assess associations between each pollutant and each NTD phenotype while accounting for potential within-group correlation. Restricted cubic splines with four knots were fitted to the logistic regression models; these knots were used to determine cut points for five levels of pollutants. Because levels of BTEX were very highly correlated, multiple pollutant models were not assessed. After adjusting for year of birth, maternal race/ethnicity, and parity, xylene exposure was consistently associated with non-significant increases in risk of spina bifida and anencephaly.

Taskinen et al. (1989) conducted a nested case-control study to investigate the effects of paternal exposure to xylene and five other organic solvents on pregnancy outcomes of wives of workers who had been biomonitored for solvent exposure. A case was a woman who had a SAB in 1973-1983 or a child with a malformation in 1973-1982. Controls were age-matched to cases at ratios of 3:1 for SAB and 5:1 for malformations. The final study sample included 120 SAB cases and 251 controls and 25 malformation

cases and 96 controls. Questionnaires were mailed to both spouses to obtain detailed data on occupational exposures during the year of conception, earlier employment, chronic diseases, smoking, and alcohol consumption. Wives were also asked for information on pregnancy history, heavy lifting, and febrile diseases. Exposure classification was based mainly on job descriptions and reported solvent use. The authors used conditional logistic regression for individually matched data. The OR (CI) for likely paternal exposure to xylene and SAB was 1.8 (1.1-3.2) when no potential confounders were included in the model. However, in analyses adjusted for confounding, no category of xylene exposure was statistically significantly associated with SAB; each of the adjusted ORs associated with the three levels of exposure was less than 1.8. In the discussion, the authors state xylene does not significantly increase the OR for SAB. The unadjusted OR (95% CI) for xylene and malformations was 1.6 (0.4-5.7). No adjusted analyses were reported for malformations, and no significant associations were reported for malformations and any of the solvents examined.

Taskinen et al. (1994) conducted another case-control study to further elucidate the possible risks of SAB, malformation, and reduced birth weight associated with maternal laboratory work. Subjects were 20-34 year old women sampled from three sources of occupational data. A SAB case was defined as a woman treated for a single SAB during the study period and a malformation case was a woman with a child registered in the Finnish Register of Congenital Malformations. Two controls for each SAB and four controls for each malformation were selected from women who had no registered SABs and had given birth to a child with no registered malformation, and matched to cases by age and year. Subjects provided information on occupational exposures, potential confounders, and child's sex and birth weight by mailed questionnaire. Exposure to individual chemicals was assessed based on questionnaire data. Conditional logistic regression for individually matched data was used to estimate ORs for SAB and malformations. The final SAB study population included 206 cases and 329 controls. SAB was associated with frequent (≥3 days/week) exposure to xylene in the first trimester: OR 3.1 (1.3-7.5), adjusted for employment, smoking, alcohol consumption, parity, previous miscarriages, failed birth control, and febrile disease during pregnancy. The malformation study included 36 cases and 105 controls, and reported no associations with occupational exposures to xylene. Birth weight was analyzed using linear regression. Taskinen et al. report a significant association between increased birth weight and "exposure at the most 2 days a week to xylene," but provide no data or measures of association.

Axelsson et al. (1984) studied the relationship between laboratory work, particularly exposure to solvents, and miscarriage, malformation, and birth weight among female employees at the University of Gothenburg. The authors contacted 811 laboratory workers, of whom 556 were eligible and completed a mailed questionnaire concerning pregnancy outcomes, occupational exposure to solvents, and potential confounders. Xylene was specifically named in the questionnaire. Additional information was obtained from birth and malformation registries. The authors observed no differences in miscarriage rates associated with exposure to solvents, including xylene. The prevalence of malformation among births to women exposed to solvents was similar to

that among unexposed women, and no results for xylene were reported. No results for birth weight and xylene were reported.

Lindbohm et al. (1990) conducted a case-control study to examine the association between SAB and maternal occupational exposure to different types of solvents. Data on Finnish women who were biomonitored for xylene or any of five other solvents were linked with hospital, clinic, and registry data to identify pregnancies and SABs. For each case, defined as a woman who had a SAB, the authors attempted to select three age-matched controls from women who had never had a registered SAB or child with a malformation. There were 73 cases and 167 controls. Mailed questionnaires were used to collect detailed data on first trimester occupational exposure and other potential risk factors for SAB. Researchers classified likelihood and level of exposure by using each woman's occupation, work description, reported use of solvents, and biological exposure measurements when available (only 5% of workers had been measured during the first trimester). The authors used logistic regression for individually matched data to estimate ORs. Five cases and seven controls were exposed to xylene. The OR for xylene and SAB was 1.3 (0.4-4.5), adjusted for previous SAB, parity, smoking, use of alcohol, and exposure to other solvents.

Windham et al. (1991) conducted a case-control study to examine the risks of SAB associated with solvent exposure defined on different levels, including occupational and specific solvent exposure. Cases were women 18 years or older who had a SAB by 20 weeks gestation, for which a pathology specimen was submitted to one of 11 hospital laboratories in Santa Clara County. For each case, the authors selected two controls with a live birth, frequency matched by last menstrual period and hospital. The sample included 626 cases and 1,300 controls. Respondents were asked for detailed information about jobs and occupational exposure to 10 specific solvents, solvent-containing products, and other solvents and degreasers during the first trimester. Women were also asked about non-occupational use of eight solvent-containing products and any other solvent. Stratified analysis was used to assess effect modification and confounding, and confounders were controlled in logistic regression models for use of any solvents and for solvent classes. Nine cases and 12 controls reported exposure to xylene. The unadjusted OR for xylene and SAB was 1.6 (0.66-3.8). For xylene exposures that involved skin contact, odor, or symptoms, the OR was 1.6 (p=0.23) and the OR for lower intensity exposure was 1.5 (p=0.52). No adjusted OR for xylene was reported. Fetal growth restriction was a secondary outcome of interest in this study; however, no results for xylene were reported.

Swan et al. (1995) conducted a retrospective cohort study to examine occupational exposures in semiconductor manufacturing and risk of SAB. The authors used company records to identify 506 current and 385 former female employees who became pregnant while working at one of 14 semiconductor companies. Job activities at conception and first trimester exposures to specific agents selected by industrial hygienists were used to classify exposures. Exposure scores were grouped into four levels ranging from no exposure to highest (0-3). The authors conducted logistic regression analyses and converted ORs to relative risks (RRs). Xylene was among the

seven agents identified as strongly associated with SAB: 2nd exposure level unadjusted RR = 1.95 (1.03-3.67) and 3rd exposure level RR = 2.70 (1.50-4.88), compared to no exposure. A chi-squared test for trend was significant, p=0.0008. When women at the 2nd and 3rd exposure levels were combined and compared with unexposed workers, the RRs were 2.31 (1.39-3.58) for all women, 2.72 (1.51-4.37) for women working in masking (including photolithography and etching), and 2.01 (0.42-5.86) for women working with dopants and thin film, adjusted for smoking, age, education, income, ethnicity, pregnancy history, pregnancy start year, and stress question. Due to simultaneous exposures to xylene, ethylene-based glycol ethers (EGE; also associated with SAB), and n-butyl acetate, Swan et al. state the associations between xylene and SAB may reflect EGE exposure.

Ghosh et al. (2012) conducted a retrospective cohort study examining the associations between term LBW and measured and modeled air pollution metrics associations. This study used both air monitoring data and previously developed land-use-based-regression (LUR) methods for modeling traffic-related prenatal air pollution exposures; however, LUR was not used to model xylene and will therefore not be discussed further in this summary. The authors used birth certificate data to identify women who resided in Los Angeles County (LA) and gave birth between January 1, 1995 and December 31, 2006. The authors compared term LBW (<2,500 g; n=8,181) with term normal weight (≥2,500g; n=370,922) infants. Preterm births (<259 days gestation) were excluded. Residential addresses were geocoded, and only those who resided <5 miles from an air toxics monitoring station managed by the California Air Resources Board (CARB) were included (n=415,531; 27.3% of those whose addresses could be geocoded). Air toxics, including BTEX, were measured every 12 days and were available for the entire study period from three monitoring stations. These measurements were used to estimate exposures for the entire pregnancy, second trimester (gestation days 93-185) and third trimester (days 186-birth), and the last month of pregnancy. Logistic regression was used to estimate ORs associated with unit or interquartile range (IQR) increases in exposure. Term LBW was associated with IQR increases in third trimester exposure to (m+p)-xylenes (OR=1.03, CI 1.01-1.06) and o-xylene (OR=1.03, CI 1.01-1.05), adjusted for maternal age, race/ethnicity, education, parity, gestational age (weeks), and gestational age squared. BTEX compounds were strongly inter-correlated, with Pearson correlation coefficients ranging from 0.79 to 0.92. Exposures to xylenes, benzene, and toluene in the last month of pregnancy were also associated with increased odds of term LBW (data not reported). Given the high correlations among the BTEX compounds, the results for xylenes should be interpreted with caution.

Lehman et al. (2002) examined the association between maternal exposure to volatile organic compounds (VOCs) and the cytokine secretion profile of cord-blood T cells. The cytokine secretion profile of T cells stimulated by environmental factors accounts for allergic responsiveness. The authors randomly selected 85 children from a cohort for another study whose subjects were recruited between December 1997 and January 1999 and included only healthy full-term neonates. Within six hours of delivery, umbilical cord blood cells were stimulated, fixed in paraformaldehyde and permeabilized, and stained against the T cell surface antigen CD3 and human cytokines

interferon-γ-producing (IFN-γ) type 1 T cells, tumor necrosis factor-α- producing (TNF-α) T cells, interleukin-2-producing (IL-2) T cells, and interleukin-4-producing (IL-4) type 2 T cells. The authors measured VOCs in the infants' homes for four weeks after birth. VOC concentrations >75th percentile were designated as elevated. Information on possible sources of VOC exposure and family atopy history was obtained from parents. Elevated (m+p)-xylene was significantly associated with cytokine-producing cord blood T-cells in unadjusted analyses. The median IFN-γ was 3.0 for (m+p)-xylene below the 75th percentile, and 4.8 for m, p-xylene above the 75th percentile ($p<0.05$). The median IL-4 was 0.71 for (m+p)-xylene below the 75th percentile, and 1.37 for (m+p)-xylene above the 75th percentile ($p<0.05$). No significant associations were observed for o-Xylene in unadjusted analyses. In multivariate logistic regression analyses that included all VOCs that showed associations in crude analyses, adjusting for maternal smoking during pregnancy, family atopy history, and gender, xylenes were not associated with cord blood cytokine-producing T cells.

Table 6. Human Developmental Studies

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Lupo et al., 2011 Texas 1999-2004 Objective: To assess the association between ambient BTEX and NTDs (spina bifida and anencephaly). Benzene was of primary interest.	Case-control study Cases were live births, stillbirths, and electively terminated fetuses with spina bifida (n=533) or anencephaly (n=303) delivered 19992004. Closed NTDs and chromosomal anomalies or syndromes were excluded. NTD data were from the Texas Birth Defects Registry. 3,695 controls were a stratified random sample of live births w/o NTD, frequency matched to cases by year of birth at a ratio of 4:1. Mixed-effects logistic regression with restricted cubic splines and 4 knots corresponding to pollutant levels where the exposure-outcome relationship changed. The knots determined cut points for five pollutant levels.	Census tract-level estimates of ambient BTEX at mother's residence were obtained from the U.S. EPA 1999 Assessment System for Population Exposure Nationwide (ASPEN), which "takes into account emissions data, rate, location, and height of pollution release; meteorological conditions; and the reactive decay, deposition, and transformation of pollutants." The xylene levels for spina bifida analyses were (µg/m^3): 0.18-0.36 >0.36-1.10 >1.10-1.96 >1.96-3.90 >3.90-8.84 Levels for anencephaly were nearly identical.	Copollutants (correlations among BTEX ≥ 0.97): • Benzene • Toluene • Ethylbenzene Potential confounders: Infant's: • sex • year of birth • season of conception Mother's: • birth place, • race/ethnicity • age • education • marital status • parity • smoking • Census tract-level estimate of socioeconomic status (% below poverty level) from the 2000 Census	ORs and 95% CIs for xylene at the 2nd-5th exposure levels, compared to 1st, adjusted for year of birth, maternal race/ethnicity, & parity: <u>Spina bifida</u> 1.45 (0.88-2.36) 1.39 (0.85-2.27) 1.18 (0.72-1.94) 1.64 (0.90-3.01) <u>Anencephaly</u> 1.35 (0.70-2.58) 1.36 (0.71-2.60) 1.32 (0.69-2.52) 1.26 (0.56-2.85) The only significant associations were for benzene and spina bifida.	Because the correlations among BTEX were so high, no multipollutant models were assessed. Authors state that ASPEN prediction model appears to be a good surrogate for personal measurements of benzene, but do not address validity for xylene. ASPEN data were only available for 1999. Authors state the sources of hazardous air pollutants were unlikely to change during the study.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Taskinen et al., 1989 Finland 1973-1983 for SAB and all controls; 1973-1982 for congenital malformations. Objective: To examine the effects of paternal exposure to organic solvents on pregnancy outcome.	Nested case-control Source population: male workers ever biomonitored for organic solvent exposure by the Finnish Inst. of Occupational Health, 1965-1983. Authors identified workers' wives and children through the population register. **SAB**: 120 cases ascertained through hospitals and outpatient clinics, 251 controls matched on maternal age. **Malformations**: 25 infants with malformations, 96 controls. Cases ascertained from Finnish Register of Congenital Malformations Conditional logistic regression	Husbands and wives completed questionnaires to provide detailed information about employment and workplace exposures during the year of conception. Solvent exposures were classified as: • Unexposed • potentially exposed • likely exposed Likely solvent exposures were further classified: • high/frequent • intermediate • low/rare Biomonitoring data included urine concentrations of methylhippuric acid for xylene, but because few measurements were taken at times relevant to the study pregnancies, measurements were used only to support exposure assessments based on questionnaire data.	Authors obtained the following information on potential confounders: Frequency of exposure to gases, vapors, dusts, fumes, and other chemicals, or radiation at work; information on earlier employment, chronic diseases, smoking, and alcohol consumption Wives were also asked for information on occupational and lifestyle exposures, as well as pregnancy history, heavy lifting and febrile diseases.	**SAB: OR (CI) for likely paternal exposure to xylene: 1.8 (1.1-3.2), without adjustment for potential confounders, including exposure to other solvents.** ORs (95% CI) for SAB and likely paternal exposure, by exposure level, adjusted for potential paternal exposure to the solvent, likely paternal exposure to other organic solvents and dusts, maternal exposure to solvents, maternal heavy lifting, and history of SAB: - Low/rare 1.2 (0.4-3.3) - Intermediate 1.7 (0.7-4.2) - High/frequent 1.6 (0.8-3.2) <u>Malformations</u>: Unadjusted OR and CI for likely paternal exposure to xylene: 1.6 (0.4-5.7)	Only 11 subjects were likely exposed to xylene without other solvents. Authors state that the small number of unexposed cases hindered many of the relevant comparisons. E.g., 14 of 120 SAB cases were classified as unexposed to solvents. Authors note that workers were also exposed to solvents other than those mentioned. The recall period for the earliest pregnancies was 14 years.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Taskinen et al., 1994 Finland Pregnancies in 1973-1986. Objective: to assess possible risks of spontaneous abortion SAB, congenital malformations , and reduced birth weight associated with maternal work in a laboratory and to identify individual chemical or physical agents potentially harmful to pregnancy.	Case-control Subjects were 20-34 year old women identified through three sources of occupational data Outcome data: •Hospital Discharge •Database of SABs treated at outpatient clinics •Finnish Register of malformations. SAB: 206 cases, 329 controls Malformations: 36 cases, 105 controls Birth weight: data not reported Conditional logistic regression for SAB and malformations; linear regression for birth weight	Subjects provided information on occupational exposures and covariates by mailed questionnaire. Two occupational hygienists who were unaware of the pregnancy outcomes assessed the overall exposure to organic solvents based on self-reported work tasks and use of solvents. Exposure was considered rare if a chemical was handled 1-2 days/week and frequent if handled ≥ 3 days/week.	Analyzed separately: • various specified organic solvents, including toluene and formalin • solvent groups, including aliphatic, aromatic, and halogenated • carcinogens • radioactive chemicals • cytostatic drugs • scintillation fluids (for measuring radiation) • pesticides • metal compounds Potential confounders: • employment, • health status, • medication, • contraception, • smoking, • 1st trimester alcohol use, • pregnancy history, • birth weight, • child's sex.	Adjusted ORs for SAB and xylene exposure in the first trimester: • 1-2 days/wk: 1.3 (0.7-2.5) • **≥ 3 days/wk: 3.1, (1.3-7.5), adjusted for employment, smoking, alcohol use, parity, previous miscarriages, failed birth control, and febrile disease in pregnancy.** No association between xylene and malformations. Authors report a **significant association between increased birth weight and exposure at most 2 days a week to xylene** (data or statistics not provided).	Simultaneous exposure to multiple solvents was common; only 2 cases and controls were exposed to xylene alone. Excluding women with multiple SABs might have introduced a bias toward the null if occupational solvent exposures increase risk of SAB. The authors provided few details concerning the malformation analyses, and no data for the birth weight analyses. 1st trimester exposures may not be adequate to assess risks associated with birth weight.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Axelsson et al., 1984 Sweden Women who worked in laboratories in 1968-1979 Objective: To study the relationship between laboratory work, particularly exposure to solvents, and pregnancy outcome (miscarriage, congenital malformations, birth weight)	Retrospective cohort Authors contacted 811 women who had worked in University of Gothenburg laboratories. 556 completed a mailed questionnaire concerning occupational exposures, induced abortions, ectopic pregnancies, miscarriages, and potential confounders. Additional information from the Swedish registers of birth and malformations. Rates and stratified analysis for miscarriage, multiple regression for birth weight.	Occupation during pregnancy and occupational exposure to solvents, including xylene and 6 other solvents during pregnancy were assessed by mailed questionnaire.	Smoking, medication use, diseases during pregnancy, pregnancy number, age, year of pregnancy, previous miscarriage, work with x-rays or radioactive isotopes, heavy lifting, stress, and shift work	Miscarriage rates were not associated with frequency of exposure to solvents or type of solvent, including xylene. The miscarriage rate [(miscarriages) ÷ (miscarriages + births + induced abortions)] among women exposed to xylene during the first trimester was 10.3% (excluding pregnancies during shift work) No other results specific to xylene, and no significant associations with solvents in general, were found for malformations or birth weight.	Length of recall period is not reported, but was at least 5 years for women who worked in laboratories in 1968 (the birth register started in 1973).

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Lindbohm et al., 1990 Finland Pregnancies between 1973-1983 Objective: To investigate whether maternal exposure to several types of organic solvents is associated with SAB.	Case-control Data on women who were biomonitored for xylene or five other solvents were linked with hospital, clinic, and registry data to identify pregnancies and SABs. A case was a woman who had a SAB. For each case, the authors attempted to select three age-matched controls from women who had never had a registered SAB or child with a malformation. There were 73 cases and 167 controls. Logistic regression for individually matched data	Mailed questionnaires were used to determine whether the woman handled or performed specific tasks with solvents, and how frequently, during the 1st trimester. Only 5% of workers had been biomonitored during the 1st trimester and the measurements reflected short-term exposures. Likelihood and level of exposure were therefore classified mainly based on occupation, work description, reported solvent use, with biological exposure measurements supporting the information when available.	Information on heavy lifting at work or home, pregnancy history, work history, health, and smoking and alcohol use during the first trimester was also collected in the questionnaires.	Five cases and seven controls were exposed to xylene. The OR for xylene was 1.3 (0.4-4.5), adjusted for previous SAB, parity, smoking, use of alcohol, and exposure to other solvents.	The biomonitored levels of urinary methylhippuric acid for xylene were low, with no results exceeding the Finnish Threshold Limit Value (10 mmol/l).

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Windham et al., 1991 Santa Clara County, California June 1986-February 1987 Objective: To examine the risk of SAB associated with solvent exposure. Fetal growth restriction was also examined, but not reported for xylene exposure.	Case-control A case was a woman ≥ 18 yrs who had SAB, for which a pathology specimen was submitted to 1 of 11 hospital laboratories. For each case, 2 controls who had a live birth were selected from county residents and frequency matched by last menstrual period and hospital. 852 cases, 1,618 controls were interviewed by telephone about the entire pregnancy (cases) or the first 20 weeks (controls). Stratified analysis was used to assess effect modification and confounding. Logistic regression	Phone interviews were used to obtain detailed information for up to 2 jobs, exposure to 10 specified solvents/solvent products and other solvents or degreasers in the 1st trimester, duration and frequency, skin contact, odors, symptoms (headaches, dizziness, forgetfulness), workplace ventilation, use of masks and respirators, and mixing or cleaning up solvent spills. Trade names were solicited to verify solvent content. Women were also asked about non-occupational use of solvents. Solvents were classified in 5 groups, including aromatic.	Maternal age, race, prior fetal loss, education, smoking, average hours worked, and interviewer-rated data quality	Xylene exposure was reported by 9 cases and 12 controls. The unadjusted OR for xylene and SAB was 1.6 (CI 0.66-3.8). The unadjusted OR for SAB and xylene exposures that involved skin contact, odor, or symptoms was 1.6 (p=0.23) and the OR for lower intensity exposure was 1.5 (p=0.52). No adjusted OR for xylene was reported.	Control of confounding was limited by the small numbers of subjects when specific exposures were examined.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Swan et al., 1995 U.S. 1986-1989 Objective: examine relationship between exposures in semiconductor manufacturing and risk of SAB. SAB was defined as a pregnancy terminated by 20 wks, except ectopic and molar pregnancy, and elective termination.	Retrospective cohort 18-44 year old women who became pregnant in 1986-1989 while working at 1 of 14 semiconductor companies. Current and former employees, of whom 189 were exposed to xylene and 683 were unexposed Outcome measure was SAB rate = SABs ÷ (live births + SABs + stillbirths). Logistic regression was used to estimate ORs, and ORs were converted into RRs.	Industrial hygienists selected specific agents and groups of chemicals based on evidence for reproductive toxicity and prevalence of exposure. Exposure scores were calculated for each employee and agent based on self-reported work activities and hours during the 1st trimester. For fabrication plant (fab) workers, exposure assessment was also based on the industrial hygienists' evaluations of each fab. Exposure scores were categorized from 0 (none) to 3 (highest). Chemical agents were also examined in functionally similar subgroups, such as photoresist and developer solvents (PDS; includes xylene). Referent groups were workers who were not exposed to a given agent or group of agents.	Workplace stress, other known or suspected risk factors for SAB, including age, smoking, ethnicity, pregnancy history, income and education, and year of pregnancy.	Xylene was among 7 agents strongly associated with SAB. Unadjusted RRs (CI) and no. SABs/exposed, by xylene exposure level: 0: referent, 79/683 1: 1.04 (0.61-1.77), 14/117 **2: 1.95 (1.03-3.67), 9/40** **3: 2.70 (1.50-4.88), 10/32** **RRs (CI) for xylene levels 2-3, vs. 0, adjusted for smoking, age, education, income, ethnicity, pregnancy history, year, stress:** **- all women 2.31 (1.39-3.58)** **- workers in masking (vs. non-fab, excluding those working with dopants and thin film) 2.72 (1.51-4.37)** - workers in dopants and thin film (vs. non-fab, excluding masking) 2.01 (0.42-5.86)	Because most women who worked in fabs were exposed to multiple, highly correlated agents, the authors were unable to model all agents simultaneously. All women exposed to n-butyl acetate (nBA) were also exposed to xylene, and most women exposed to xylene and nBA were also exposed to ethylene-based glycol ethers, which were also associated with SAB.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Ghosh et al., 2012. Los Angeles County (LA) January 1995-December 2006 Objective: to examine whether measured and land-use-based-regression (LUR) modeled air pollution metrics showed consistent associations with term LBW, indicating a role for traffic exhaust exposures.	Retrospective cohort Authors obtained birth certificate data for women who resided in LA between January 1995 and December 2006 (n=1,745,754). Term LBW (≥260 days gestation and <2,500 g; n=8,181) and term normal weight (≥2,500g; n=370,922) infants were compared. Births with recorded defects, missing or gestational age >320 days, birth weights <500 g or >5,000 g, or multiple gestations were excluded. Only those who resided <5 miles from at least 1 of 4 California Air Resources Board (CARB) air toxics stations were included in the study (n=415,531; 27.3%). Logistic regression	Residential addresses from birth certificates were geocoded and overlaid with the geocoded locations of LA air toxics monitoring stations managed by CARB. Air toxics, including BTEX, were measured every 12 days for the entire study period by 3 CARB air monitoring stations and for 2000-2006 by a 4th air monitoring station. Air toxics measurements were used to estimate average exposures for the entire pregnancy, 2nd (days 93-185) and 3rd (days 186-birth) trimesters, and the last month of pregnancy. LUR models were not used for BTEX.	Authors adjusted for the following confounders identified in their previous studies: - maternal age - race/ethnicity - education - parity - gestational age - (gestational age)^2 Mother's birthplace and a Census-based measure of socioeconomic status were also evaluated for confounding, but not included in final models.	The overall prevalence of term LBW was 2.2%. BTEX compounds were strongly inter-correlated, with correlation coefficients 0.79-0.92. Due to small case counts and unstable estimates, results from the 4th air monitoring station were not reported. **Term LBW was associated with an IQR increase in 3rd trimester exposure to (m+p)-xylenes (OR=1.03, CI 1.01-1.06) and o-xylene (OR=1.03, CI 1.01-1.05).** **Exposures to xylenes, benzene, and toluene in the last month of pregnancy were also associated with term LBW (data not reported).**	Due to the high correlations among the BTEX compounds, the results for xylenes must be interpreted with caution. Regarding misclassification due to residential mobility and the use of birth certificate addresses, authors state that LA women who move during pregnancy typically stay in the same neighborhood. When data were restricted to women within 3 miles of monitoring stations, associations were somewhat stronger but large overlap in CIs, suggesting some misclassification due to residential distance from monitoring stations. Ghosh et al. also note that excluding preterm babies introduced selection bias because excluded preterm growth-restricted infants may have had greater exposures to air pollution.

Table 6. Human Developmental Studies (continued)

Study	Methods	Exposure Assessment	Covariates	Results	Comments
Lehman et al., 2002 Leipzig, Germany December 1997-January 1999 Objective: To investigate the association between VOC exposure and alterations of the cytokine secretion profile of cord-blood T cells.	Cross-sectional 85 healthy, full-term neonates whose mothers did not suffer autoimmune diseases or infectious disorders during pregnancy, randomly drawn from an ongoing prospective cohort study of maternal exposure to VOCs and immune status at birth. Umbilical cord blood samples were taken at delivery and T cell function analyzed using intracellular cytokine staining. Mann-Whitney U-tests and logistic regression	Parents answered questionnaires on possible sources of VOC exposure (including painting, flooring, and smoking in the home) and family atopy history. VOCs were collected by continuous passive sampling in children's homes for 4 weeks after birth. Concentrations above the 75th percentile were considered elevated.	Maternal smoking during pregnancy, family atopy history, gender	**Elevated (m+p)-xylene was significantly associated with cytokine-producing cord-blood T cells in <u>unadjusted</u> analyses: the median number of IFN-γ T cells was 3.0 for (m+p)-xylene < 75th percentile, and 4.8 for (m+p)-xylene > 75th percentile (p<0.05).** **The median number of interleukin-4-producing (IL-4) T cells was 0.71 for (m+p)-xylene < 75th percentile, and 1.37 for (m+p)-xylene > 75th percentile (p<0.05).** No significant associations were observed for o-xylene in unadjusted analyses. In multivariate analyses, xylenes were not associated with cord blood cytokine-producing T cells.	Authors note that the clinical relevance of altered cytokine secretion profile of cord-blood T cells is not known.

E.1.2. Related Human Developmental Studies

Aguilar-Garduño et al. (2010) studied the relationship between parental occupational exposure to organic solvents and risk of anencephaly in Mexico. Eligible cases were 151 live births and fetal deaths with anencephaly that had reached ≥20 weeks gestation. For each case, a live-born control without apparent congenital malformations was selected from the same maternity service and birth date. Parents were interviewed about five-year occupational history, exposure to chemical agents in the residential area, sociodemographic characteristics, behaviors, and other potential confounders. A food frequency questionnaire was also administered to the mothers. Workers who reported use of organic solvents or worked in an occupation linked to exposure to organic solvents were considered exposed. No mothers of controls were exposed to organic solvents during the acute risk period (ARP; defined as the period from three months before to one month after the date of the mother's last menstruation), so the risk associated with maternal exposure to organic solvents in the ARP could not be estimated. Occupational exposure to organic solvents in the past five years was associated with increased odds of having a child with anencephaly; adjusted OR = 9.22 (CI 1.97-43.17) for maternal exposure and OR = 2.16 (1.08-5.32) for paternal exposure. The adjusted OR for anencephaly and exposure of at least one parent to organic solvents during the ARP was 2.97 (1.36-6.52).

Chevrier et al. (2006) assessed the association between maternal occupational exposure to organic solvents and the risk of non-syndromic oral clefts. Cases were defined as children diagnosed with non-syndromic cleft lip with or without cleft palate (CL/P; n=164) or cleft palate only (CP; n=76) and were recruited during their initial hospitalization for maxillofacial surgery. Matched controls (n=236) were recruited from the same hospitals and two additional neighboring maternity hospitals, where they were being treated for conditions other than birth defects, cancer, or genetic diseases. A chemist assessed each mother's exposure to 22 classes of agents. Each exposure was modeled separately by unconditional logistic regression. Exposures to petroleum solvents were associated with CL/P: petroleum solvents overall, OR = 3.64 (CI 1.5-8.8); very low to low exposure, OR = 3.21 (1.1-9.3); and medium to high exposure, OR = 4.60 (1.1-19.2); linear trend $p<0.01$.

Garlantézec et al (2009) studied the effect of maternal occupational solvent exposure on the risk of malformations in a population-based prospective cohort study. Women in Brittany, France were recruited at prenatal care visits and followed up from 16 weeks gestation through the end of pregnancy. The 3,005 subjects completed questionnaires about occupational exposures and potential confounders. A job-exposure matrix (JEM) was also used to assign probability of solvent exposure to a combination of occupation code and industrial activity code for each subject. There were 3,041 pregnancies (36 twin pregnancies), 118 had at least one malformation (including 14 elective abortion); 84 were classified as major and 34 as minor malformations. The authors found a significant association between risk of major non-chromosomal, non-genetic malformations and maternal solvent exposure: OR = 2.48 (1.4-4.4) for self-reported exposure and OR = 3.48 (1.4-8.4) for exposure according to JEM, with dose-response

relationships (p=0.002 for self-report and p=0.005 for JEM). No association was observed for either chromosomal or genetic malformations.

Holmberg and Nurminen (1980) examined parental occupational factors as risk factors for congenital central nervous system (CNS) defects, including anencephaly, hydrocephaly, spina bifida, microcephaly, and other CNS defects. Cases (n=120) were identified through the Finnish congenital malformation registry and from death certificates for stillbirths and infant deaths. Controls were the mothers who delivered immediately before a case mother in the same district. Information on occupational factors was limited to mother's occupation and whether she continued working during pregnancy, and father's occupation. Additional information on mothers' exposures at work was obtained in interviews, by consultations with a chemist and an industrial hygienist and, if necessary, by contacts with or visits to the industries concerned. The OR for maternal occupational solvent exposure and CNS defect was 4.2 (CI or p-value not reported), and the combined effect of solvent and dust exposures appeared to be synergistic.

Khattak et al. (1999) evaluated pregnancy outcomes following maternal occupational exposure to organic solvents in a prospective cohort study in Toronto. The primary outcome of interest was the rate of major malformations and other outcomes of interest were rates of minor malformations, miscarriages or therapeutic abortions, premature birth, birth weight, gestational age at delivery, and fetal distress or other neonatal complications. Study participants were identified through a prenatal counseling service. Women who were occupationally exposed to organic solvents (n=125) were matched to unexposed (defined as exposed to non-teratogenic agents only) women on age, gravidity, and smoking and drinking status. Detailed information on chemical exposures during pregnancy was collected during the initial assessment meeting and a postnatal assessment was obtained six to nine months after the expected delivery date. There were 13 major malformations, of 13 types, among the exposed and one among the unexposed, RR = 13.0 (CI 1.8-99.5).

Testud et al. (2010) conducted a prospective cohort study to determine if occupational and toxicological risk assessment would influence pregnancy outcomes among women exposed to organic solvents in the workplace. Outcomes of interest were miscarriage, malformations, birth weight, and gestational age at delivery. Pregnant women who requested toxicological advice or whose physicians requested toxicological advice by calling the Lyon Poison Center were recruited for the study. Women who were exposed to organic solvents in various occupations since conceiving were considered exposed (n=206). Each exposed woman was matched with a woman who had called the poison center because of an exposure to non-embryotoxic agents. An occupational physician and medical toxicologist interviewed each woman to assess exposure. Birth outcome was obtained from the woman's physician. There were 197 live births, including 3 sets of twins, in each of the exposed and unexposed groups. No significant differences between the groups were observed for miscarriage, malformations, birth weight or gestational age at birth. Although the authors list xylene among the solvents of concern for this study, no results specific to xylene are reported.

Wang (1994) examined pregnancy syndromes, menstrual cycles, and pregnancy outcomes among 161 women who were exposed to BTX through their work in the painting industry, compared with 154 unexposed women who worked in hotels and were matched to exposed women by age and other characteristics. The average air xylene concentration in 29 samples was 36.8 mg/m^3. A larger proportion of exposed women's pregnancies ended in abortion. The study is reported in Chinese and with very little detail.

Magnusson et al. (2006) investigated adverse pregnancy outcomes in the offspring of men employed in biomedical research laboratories at four Swedish universities during 1970-1989. Pre-or periconceptional (90 days prior to conception or during gestation) laboratory work was considered an exposure. More specific laboratory exposures were assessed using questionnaires completed by research group leaders for 59% of births. Most periconceptionally-exposed pregnancies were exposed to more than one class of agent, such as organic solvents, carcinogens, or radioactive isotopes. ORs (CI) for birth outcomes and paternal periconceptional exposure to organic solvents, adjusted for father's and mother's age and maternal occupation, smoking, chronic diseases, height, and gestational weight gain, were as follows:

- low birth weight (<2,500 g; n=14) OR = 1.0 (0.3-3.2)
- high birth weight (>4,000 g; n=111) OR = 0.5 (0.3-1.0)
- small for gestational age (n=10) OR = 1.5 (0.4-5.4)
- large for gestational age (n=20) OR = 0.8 (0.2-3.3)
- preterm birth (<37 weeks; n=25) OR = 0.9 (0.4-2.4)
- post-term birth (≥42 weeks; n=62) OR = 1.1 (0.6-2.0)

Work with organic solvents was associated with a 44 g (CI -11-99) increase in birth weight.

Ha et al. (2002) conducted a retrospective cohort study to examine the association between birth weight and maternal and paternal exposures to organic solvents at BYPC, a large petrochemical corporation in Beijing. The major occupational exposures are benzene, toluene, styrene, and xylene, all maintained below 1 ppm as an 8-hr time-weighted average. The cohort consisted of 1,222 mother-father-infant triads: mothers who were employees of BYPC and had had a live birth at the staff hospital, fathers who also worked at BYPC, and their infants. One hundred eleven mothers and 126 fathers were exposed to organic solvents. Occupational exposures and other information were obtained by separate interviews of women and men. Maternal clinical data and birth outcomes were recorded by a trained nurse. The mean birth weight was 3,347.0 g for children of exposed mothers and 3,424.0 g for the unexposed mothers. When adjusted for infant gender, maternal pre-pregnancy body mass index and age, and paternal height and education, maternal exposure was associated with a decrease in birth weight of 79.0 (CI 1.9-156.0) g. If the model adjusted for paternal exposure, the decrease in birth weight associated with maternal exposure was similar: 81.7 (CI 3.1-106.3) g.

Aguilera et al. (2009) investigated the effect on birth weight of prenatal exposure to motor vehicle exhaust pollution (nitrogen dioxide [NO_2] and BTEX), using geographic information systems models. The cohort consisted of 570 women who visited the public health center of Sabadell, Spain, in the 12th week of pregnancy. To estimate individual exposures to NO_2 and BTEX, the authors used NO_2 and BTEX data taken over three one-week periods from 57 sampling sites. Land coverage, topography, population density, roads, and distances to local sources of pollution as predictor variables were then fitted to the NO_2 and BTEX data to develop land use regression (LUR) models to predict outdoor pollution levels at the women's residential addresses. BTEX compounds were highly correlated ($r > 0.75$). The sum of the BTEX compounds was used to assess the relationship with birth weight. BTEX exposure was not significantly associated with birth weight, except for exposures in the second trimester among women who spent <2 hour/day in nonresidential outdoor environments. For these women, an interquartile range increase in BTEX was associated with a decrease in birth weight of 137.3 (22.0–-252.6) g, adjusted for child's sex, gestational age, season of conception, parity, maternal education, maternal smoking during pregnancy, maternal height and pre-pregnancy weight, paternal height, and exposure in the other trimesters. The authors suggest that the difference in birth weight was significant for this group of women because there may have been less exposure misclassification in the LUR estimates. Exposure to BTEX during the whole pregnancy was also associated with a statistically significant decrease in birth weight of 76.6 (CI 7.0-146.3) g.

In a related study, Aguilera et al. (2010) investigated the relationship between prenatal exposure to vehicle exhaust and longitudinally measured fetal growth parameters from ultrasound examinations in the same cohort studied by Aguilera et al. (2009). The 2010 study uses the same exposure and covariate data collection as the 2009 study. The following fetal parameters were recorded at routine fetal ultrasound examinations in each trimester: femur length, head circumference, biparietal diameter (BPD), abdominal circumference, and estimated fetal weight. LUR was used to estimate individual exposure to traffic-related air pollution. Aguilera et al. reported a 4.82 (CI 0.45-9.12)% decrease in BPD in weeks 20-32 associated with an interquartile range increase in BTEX in weeks 1-12, adjusted for season of conception, parity, maternal education, maternal smoking, and pre-pregnancy weight. The authors state that because BTEX and NO_2 were estimated with LUR models, they consider the pollutants as markers of vehicle exhaust toxins rather than potential causative agents by themselves.

Yang (1997) reported on a survey of occupational diseases among female workers in Liaoning Province, China. The study population included 3,248 women exposed to BTX through work in the rubber, leather, chemical, paint and insulation industries. The unexposed were 7,247 women working in the postal service, retail, and services. The two groups were similar in terms of marital status, age, education, years of work, passive smoking and other measures of socioeconomic status. The following were more prevalent among exposed than unexposed women: abortion signs (0.6% vs. 0.09%; $p<0.005$), abortion (6.2% vs. 3.3%; $p<0.005$), preterm birth (1.6% vs. 0.5%), and delayed birth (0.4% vs. 0.03%). The offspring of exposed women were also more likely

to experience the following problems: low birth weight (8.4% vs. 3.0%; $p<0.005$), mental retardation (7.1% vs. 0.6%; $p<0.005$), and newborn death (5.7% vs. 2.0%; $p<0.005$).

Magnusson et al. (2006) examined the association between prenatal occupational exposures, especially organic solvents, and atopic diseases in childhood. Outcomes of interest in this prospective cohort study were asthma, hay fever, atopic eczema, and wheezing. Mothers who visited midwife centers at approximately 36 weeks gestation in Odense and Aalborg, Denmark were given a self-administered questionnaire about lifestyle, work, and "other social conditions." Assessment of occupational exposure to organic solvents was based on job titles reported in questionnaires and likelihood of exposures above the Danish threshold limit value (TLV). The authors contacted the children and their parents 14-18 years later to ascertain atopic diseases. The OR for exposure to organic solvents above the TLV during pregnancy and hay fever, with six exposed cases, was 2.8 (1.1-7.5), adjusted for socioeconomic group; maternal education, age, parity, and smoking; postnatal parental smoking; and breast feeding. The OR for asthma diagnosis was 1.8 (0.6-5.6).

Windham et al. (2006) did a case-control study in the San Francisco Bay area to explore associations between autism spectrum disorders (ASD) and environmental exposures during pregnancy or early life. Cases were 284 children with ASD born in 1994 who were identified through an active surveillance system and represented an approximate population-based series. Controls were selected from birth certificates for the same counties and matched to cases by sex and month of birth at a 2:1 ratio. Exposure assessments were based on annual census tract level estimates of hazardous air pollutant (HAP) concentrations for 1996 at the mother's residence at time of delivery. Concentrations from all sources were summed for each compound. The mean ± SD concentrations of xylene were 3.77 ± 1.68 $\mu g/m^3$ for cases and 3.63 ± 1.46 $\mu g/m^3$ for controls. Aromatic solvents were highly correlated with one another (r = 0.89-0.99) and with polyaromatic hydrocarbons and manganese. The OR (CI) for ASD and combined aromatic solvent exposure in the third quartile (using the control distribution to determine cut points) compared to first and second quartiles was 0.84 (0.59-1.20), adjusted for maternal age, education, and child race. For fourth quartile exposures, the adjusted OR was 1.15 (0.80-1.65).

Laslo-Baker et al. (2004) evaluated occupational exposure to organic solvents during pregnancy as a risk factor for adverse neurodevelopmental outcomes in children. Subjects were recruited through an information and counseling program on the risk of drugs, chemicals, radiation, and infection during pregnancy and lactation. Thirty-two women occupationally exposed to organic solvents for at least eight weeks, commencing in the first trimester were considered exposed. Women with non-teratogenic exposures were considered unexposed and were matched to exposed women on age, child sex, socioeconomic status, and cigarette use. Details about occupational exposures were recorded at the time of the mother's first contact with the program and twice postnatally. A battery of neurodevelopmental and cognitive functioning tests was administered to each child and mother, and each mother completed two questionnaires assessing the child's behavioral functioning. After

adjustment for maternal IQ and education, exposure to organic solvents was the only factor that accounted for variances in digit span, information, vocabulary, recalling sentences, dexterity, and visual-motor coordination scores, and hyperactivity/impulsivity, though most associations were not significant.

A paper by Kucera (1968) reporting a study in which chick embryos were exposed to xylene vapors also reported on nine cases of sacrococcygeal agenesis, or caudal regression, among infants born in Czechoslovakia between the years 1959 and 1966. Based on questionnaire data obtained from parents of these patients, five cases were associated with exposure to organic solvents (including xylene) during pregnancy. Only one of these cases, a stillborn male infant with multiple malformations, was associated with exposure to xylene. The mother reported daily exposure to 3000 ml of xylene between the 3rd and 16th weeks of pregnancy

E.2. Animal Developmental Toxicity Studies

Fifteen studies were identified as having information on the developmental toxicity of technical or mixed xylene and/or its individual isomers in animals (Biodynamics, 1983; Hass et al., 1995; Hass et al., 1997; Hass and Jakobsen, 1993; Hudak and Ungvary, 1978; Kükner et al., 1997; Litton Bionetics, 1978; Marks et al., 1982; Mirkova et al., 1979; Mirkova et al., 1983; Rosen et al., 1986; Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary et al., 1981; Ungvary and Tatrai, 1985).

The majority of available studies assessed standard endpoints of developmental toxicity, and exposed pregnant animals to technical xylene by the inhalation route of exposure. These studies are discussed in section E.2.1. below. One study each employed the oral or dermal routes of exposure; these studies are discussed in sections E.2.4. and E.2.5. below, respectively. Four studies, discussed in section E.2.2. below, evaluated the developmental neurotoxicity of xylene. Three studies compared the effects of technical xylene with each of the individual xylene isomers, while two additional studies tested only p-xylene. Studies of individual isomers are discussed in section E.2.3. below.

An additional study, described in section E.2.6, contains related information on the effects of exposing chick embryos to xylene vapors (Kucera, 1968).

E.2.1. Developmental Toxicity: Technical Xylene by Inhalation

Nine studies provided information on endpoints of developmental toxicity for technical xylene by the inhalation route of exposure (Biodynamics, 1983; Hass and Jakobsen, 1993; Hass et al., 1995; Hass et al., 1997; Kükner et al., 1997; Litton Bionetics, 1978; Mirkova et al., 1983; Saillenfait et al., 2003; Ungvary and Tatrai, 1985). Some of these studies also provided information on the developmental toxicity of individual xylene isomers, or included data on postnatal developmental effects; that information is described under appropriate headings below.

Biodynamics (1983) conducted a one-generation reproductive toxicity study, with a teratology component. CD rats were exposed to mixed xylenes by chamber inhalation during a 131-day pre-mating period and 20 days of mating. Pregnant females continued on their treatment regimen throughout gestation and lactation, excepting for the days between GD 20 and PND 5. Exposure levels were 0 (group I), 60 (group II), 250 (group III), and 500 ppm, daily for six hrs/day. Animals exposed to the highest concentration of xylene were divided into three groups:

- 20 males and 40 females, both sexes treated (group IV)
- 10 males and 20 females, only males treated (group V)
- 10 males and 20 females, only females treated (group VI)

Twenty females from group I (sham controls) and 12 females from group IV (500 ppm) were sacrificed on GD 21 for fetal evaluations for the teratology component of the study. The remaining pregnant females from all groups were allowed to deliver normally.

No mortality was seen among maternal animals of the treated groups; among controls, two females died, while a third female was sacrificed moribund. No adverse effects were found on on maternal body weight or gross postmortem evaluation, and no clinical symptoms of toxicity were observed. Group IV females showed an increase in mean kidney weight, and increased mean corrected body weight at GD 21; mean kidney to body weight ratio showed a nonsignificant increase compared to controls.

Results for the teratology part of the study revealed no differences between control and exposed groups for the number of dams with implantations, corpora lutea per litter, implantations per litter, resorptions per litter, or live fetuses per litter. There was no effect on sex ratio, and no effect on the body weight of male fetuses. This study provided no evidence for a significant effect of treatment on the frequencies of external, internal, or skeletal variations or malformations. The only significant effect observed was a decrease in the weight of female fetuses exposed to 500 ppm xylene.

Among the litters allowed to go to term, mean litter size for the controls was only 9.6 pups per litter. Therefore, groups III and IV (60 and 250 ppm, respectively), had significantly larger litters than controls (12.5 and 12.4 pups, respectively). The reduced litter size for controls may also have affected the findings for fetal weights, as fetuses/pups tend to be larger in smaller litters (US EPA, 1991). Nonetheless, mean pup weights on PND 1 did not differ among groups.

Hass and Jakobsen (1993) exposed groups of 36 timed-pregnant Wistar rats to technical xylene at concentrations of 0 or 200 ppm. Animals were exposed for six hours per day on GD 4-20. Two thirds of the pregnant rats were sacrificed on GD 21 for evaluation of their uterine contents. Remaining animals were allowed to deliver their litters normally.

This study provided no evidence for maternal toxicity of xylene under the conditions tested. No statistically significant effects of xylene treatment were identified on

endpoints such as fetal viability, fetal weight, or sex ratio. Nor were significant effects observed in the incidences of visceral or skeletal anomalies or malformations. Some evidence was found, however for an increase in skeletal retardation among xylene-exposed fetuses, primarily consisting of delayed ossification of the *os maxilliare*.

Live-born pups in this study showed no treatment-related differences in the number of pups per litter or sex ratio. Birth weights of male pups were significantly higher among treated than control pups. The study authors noted that parameters such as gestation length and litter size did not differ between groups in this study, and therefore did not explain the birth weight differences.

Hass et al. (1995) exposed timed-pregnant rats to 0 or 500 ppm technical xylene for six hours per day on GD 7-20. This concentration and exposure period were specifically selected to avoid both maternal and embryo-fetal frank toxicity. A total of 13 control and 15 exposed litters were evaluated. No evidence was found for maternal toxicity, nor for adverse impacts of exposure on gestation. Neonatal viability and sex ratio were not affected by prenatal exposure to xylene at 500 ppm. Mean birth weight for exposed litters was reduced by approximately 6% relative to controls, but the difference was not statistically significant.

Hass et al. (1997) repeated the previous protocol (Hass et al., 1995), in order to further study the behavioral effects described in section E.2.1.2, below. Neither maternal toxicity nor evidence of embryo-fetal toxicity was observed at birth. Offspring body weights did not differ between treated and control animals during the entire study period.

Kükner et al. (1997) exposed seven pregnant Wistar rats to xylene by inhalation at a concentration of 2600 ppm for eight hours per day, seven days per week, from GD 6 through term. A control group of five pregnant animals were exposed only to clean air in the inhalation chambers. An additional group of five non-pregnant female rats were exposed to xylene under the same conditions as the pregnant animals.

Xylene treatment did not affect gestational weight gain, or weight gain of the non-pregnant females. Blood samples from pregnant rats did not reveal significant differences among groups in hemoglobin and hematocrit values. Samples of maternal liver, however, showed xylene-related increases in the activities of aspartate aminotransferase, alanine aminotransferase, and arginase, as well as an increase in levels of alkaline phosphatase.

Newborn pups exposed prenatally to xylene did not show changes in the frequencies of external abnormalities or internal gross soft tissue anomalies. Histopathological evaluations revealed no structural changes in the pancreas or kidney tissues of rat pups prenatally exposed to xylene. Histology of their livers, however, showed a number of pathological changes such as heterochromatic nuclei, vacuolization, and dilated rough endoplasmic reticulum.

Litton Bionetics (1978) exposed timed-pregnant CRL:COBS CD (SD) BR rats to airborne concentrations of xylene at 0, 100, or 400 ppm, on each of gestation days 6-15. Effective daily exposure was six hours. Animals were sacrificed on GD 20 for standard teratological evaluations.

Mean body weights or food consumption for maternal animals did not vary among groups. Necropsies of these animals did not reveal gross visceral pathology. Nor were significant differences among groups found at fetal evaluation for parameters of viability, growth, or malformation frequency. Concurrent control and historical control data "were considered to be acceptably similar."

A statistically significant increase in skeletal changes classified as "unusual" in the 400 ppm xylene group was seen only when evaluated for all fetuses, not on a per litter basis. The report offered no clear definition of what constituted an "unusual" skeletal observation. Although 10 litters in the 400 ppm xylene group were reported as having at least one affected fetus, the majority of affected fetuses were reported to have come from three specific litters. According to the authors, the fetuses from all three of these litters were considered to be small and developmentally retarded. The authors did not attribute these observations to the effect of xylene exposure.

Mirkova et al. (1983) exposed pregnant Wistar rats by inhalation to xylene at concentrations of 0, 2.31, 11.53, or 115 ppm, for six hours per day, five days per week, from GD 1-21. On GD 21 some of the animals were sacrificed for fetal evaluation, while others were allowed to deliver their litters normally. Pups were evaluated at one month and three months postnatal age.

The paper makes no mention of maternal mortality or weight gain during the study. Reporting of litter and fetal variables included findings of no effects from prenatal xylene exposure on implantation frequency, fetal viability, or resorption frequency. Effects seen at concentrations of 11.53 and 115 ppm xylene included increased post-implantation loss, decreased mean fetal weight per litter, increased percentages of total fetuses with hemorrhages, and increased frequencies of skeletal anomalies. The high concentration of 115 ppm xylene was stated to be associated with a significant increase in the incidence of visceral abnormalities, but data were not provided.

The authors also reported effects on postnatal growth and functional development in pups exposed prenatally to xylene concentrations of 11.53 and 115 ppm. Effects included reduced pup weights on PND 7 and 21, and "metabolic disturbances" in the liver, brain, myocardium, and lungs. Specifically, data from lung tissue on PND 21 showed significant increases in activity of acid phosphatase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase.

As discussed below in Section E.2.3., Saillenfait et al. (2003) compared the developmental effects of o-, m-, and p-xylene with technical xylene in rats following inhalation exposure. Technical xylene had no effect on maternal mortality, pregnancy rate, corpora lutea, implantation sites, percentages of live and dead fetuses per litter,

resorption frequency, fetal sex ratio, or the frequencies of external or internal malformations. Maternal gestational weight gainand mean fetal weights were significantly reduced at technical xylene concentrations of 1000 and 2000 ppm. Technical xylene exposure was not associated with increased frequencies of external, visceral, or skeletal anomalies.

Ungvary and Tatrai (1985) studied the effects of technical xylene and/or its individual isomers by inhalation in pregnant rats, mice, and rabbits. Test animals from each species were exposed for 24 hrs/day throughout organogenesis. Results for individual isomers are described and discussed below in section E.2.3., while results for technical xylene will be discussed here.

CFY rats were exposed to technical xylene at concentrations of 0, 58, 438, or 784 ppm, while CFLP mice and NZ rabbits were exposed to xylene concentrations of 0, 115, or 231 ppm.

Maternal mortality was seen in rats with the high concentration of technical xylene: 1/20 dams died at 784 ppm. Among rabbits, 3/10 dams died at 231 ppm technical xylene. Data on maternal gestational weight gain were reported only for rabbits, and no significant effect was observed at any concentration for technical xylene. Maternal liver weights were found to be increased in rabbits exposed to the high concentration of technical xylene.

Fetal viability in mice was not affected by technical xylene. Rats showed no effect of technical xylene on the numbers of live fetuses per group, but there was an increased percentage of dead or resorbed fetuses at the high concentration of 784 ppm. Rabbits showed the greatest sensitivity to effects of xylene on embryo-fetal mortality, with no surviving fetuses at the high concentration of 784 ppm.

Effects of technical xylene on fetal weights were seen at 784 ppm in rats, and 231 ppm in mice. Technical xylene at 115 ppm was associated with a significant decrease in the mean weight of female rabbit fetuses.

In rats exposed to technical xylene, increased percentages of fetuses were found to have evidence of skeletal retardation as compared to controls at 58, 438, and 784 ppm. At 784 ppm, increased percentages of rat fetuses were found to have minor anomalies, such as extra ribs. Mice exposed to 231 ppm technical xylene showed evidence of skeletal retardation, but no effect was observed on the percentages of minor anomalies such as extra ribs. Rabbit fetuses did not show changes in the frequencies of skeletal retardation or the presence of anomalies with exposure to technical xylene.

E.2.2. Behavioral Endpoints of Prenatal Exposure to Xylenes

Four studies looked specifically at the developmental neurotoxicity of prenatal exposure to xylene (Hass et al., 1995; Hass et al., 1997; Hass and Jakobsen, 1993; Rosen et al., 1986). Of these studies, only one looked at a specific isomer of xylene: Rosen et al.

(1986) performed behavioral tests on animals prenatally exposed to p-xylene. The other three studies used technical xylene.

Standard teratological endpoints for the experiments by Hass and Jakobsen (1993) are described above in Section E.2.1. Pregnant rats were exposed to concentrations of 0 or 200 ppm technical xylene six hrs/day on GD 4-20. Rotarod tests were performed on PND 22, 23, and 24. Rotarod time was reduced for exposed female pups on all three test days (days 22-24), but only the reductions seen on PND 22 and 23 were statistically significant. For males, Rotarod time was significantly reduced only on the second test day (day 23). The authors concluded that impairment of Rotarod performance, which was mainly significant in females, could be interpreted as an indicator of impaired motor ability in xylene-treated animals.

In a study of the effects of prenatal exposure to xylene on postnatal development and behavior in rats, Hass et al. (1995) used concentrations of 0 or 500 ppm. Maternal effects and observations on pups at birth from this study are described above in section E.2.1. Postnatal and behavioral development are discussed in this section. Pre-weaning pups were evaluated for attainment of physical developmental landmarks, such as pinna unfolding, and acquisition of reflexes. Post-weaning pups were evaluated for performance on the Open Field test, the Rotarod test, and in the Morris water maze.

While other developmental landmarks were unaffected, acquisition of the air righting reflex was delayed in exposed pups. These findings were taken by the study authors to suggest damage to neural processes required for air righting, such as vestibular function, rather than a more general developmental delay.

The Rotarod test did not detect statistically significant differences between treated and control groups. The overall trend, however, was for a greater percentage of exposed animals to fail at reaching 30 seconds on the rod, and to have a shorter mean time on the Rotarod. This effect was most marked in female offspring in the third trial on PND 26, and was taken to indicate a trend toward impairment of motor coordination and balancing abilities in exposed female offspring.

The Open Field test revealed no exposure-related differences between treated and control groups for ambulation, the percent of time spent in the center of the field, or the frequency of defecation.

In the Morris water maze test, a non-significant trend suggested that xylene-exposed animals took slightly longer to find the platform during initial test blocks. No exposure-related differences between groups were seen for memory after three weeks, or during reversal learning when the platform was moved to the opposite quadrant of the pool. Additional water maze experiments found that exposed offspring took significantly longer to find the platform following its relocation to the center of the pool. Separate analysis of each sex revealed that the exposure-based difference was significant only for female offspring. Exposed females also showed a significant increase in swimming time, but no difference in swimming speed relative to controls.

As a follow-up to their previous study (Hass et al., 1995), an additional investigation was conducted specifically to look at impaired performance in the Morris water maze test by young female rats exposed *in utero* to xylene (Hass et al., 1997). The treatment protocol was as described for the previous study. Once the delivered pups were weaned, one female pup per litter, selected as having the median body weight, was reserved for behavioral investigations.

The animals were tested in the Morris water maze at the ages of 12 weeks, 16 weeks, 28 weeks, and 55 weeks. Testing was performed during the animals' dark period, when they are normally most active.

At week 12, the xylene-exposed offspring took significantly longer than control offspring to find the platform. When the test was repeated four weeks later (16 weeks postnatal age), no difference was observed between groups whether the platform within the same position as before (memory test) or had been moved to the opposite quadrant of the pool (reversal learning). Differences between treated and control animals were revealed when the platform was moved to the center of the pool, with exposed offspring taking significantly longer to find the relocated platform. Further analysis indicated that swim speed was not different between the treated and control groups, and hence did not account for the increased latency. The xylene-exposed group showed a significantly increased length of swim path.

At the age of 28 weeks, control and treated groups did not differ significantly in water maze performance when the platform was in a position the animals had previously experienced. When the platform was moved to a new position at the rim of the pool, increased latency was observed in xylene-exposed animals. This latency was significant in the first trial of the block. When this result was broken out into consideration of swimming path length versus swimming speed, swimming path length was found to be significantly increased. Swim speed did not vary between groups.

At 55 weeks of age, treated and control groups did not differ in their ability to find the platform located in what had been the new position at 28 weeks. Treated animals did show a greater, but not statistically significant, latency in finding the platform at earlier test positions (i.e. those used at 12 weeks).

In summary, increased latencies in the Morris water maze were observed in animals prenatally exposed to xylene when tested at postnatal ages of 16, 28, and 55 weeks. These differences did not appear to be related to swim speed or other motor difficulties with swimming. On the other hand, swim path lengths of treated animals were longer in proportion to the increased latencies. By 55 weeks of age, effects were not statistically significant, suggesting at least partial reversibility of xylene-induced impairment after a long period of time. Alternatively, repeated testing may have allowed the animals to develop skills at locating the platform, helping to compensate for any organic deficits caused by prenatal xylene exposure.

In a study that used p-xylene, rather than technical xylene, Rosen et al. (1986) exposed groups of 25 pregnant Sprague-Dawley rats to 0, 807.31, or 1614.62 ppm p-xylene for six hours per day on GD 7-16. Observations on maternal animals and pup viability and growth are described in section E.2.1.3., below. The acoustic startle response test was performed on PND 13, 17, 21, and 63. The same animals were tested for locomotor activity in figure-eight mazes on PND 22 and 65. Neither tests of locomotor activity, nor the acoustic startle response showed any effect of treatment.

E.2.3. Developmental Effects of Prenatal Exposures to Individual Xylene Isomers

Three studies compared the effects of technical xylene with each of the individual xylene isomers (Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary and Tatrai, 1985). Two additional studies evaluated the developmental effects of p-xylene only (Rosen et al., 1986; Ungvary et al., 1981).

Saillenfait et al. (2003) compared the developmental effects of o-, m-, and p-xylene with technical xylene in rats following inhalation exposure. Each agent was provided at concentrations of 0, 100, 500, 1000, or 2000 ppm. The duration of exposure was six hours per day, on each of GD 6-20. Dams were sacrificed on GD 21, and their uteri removed for evaluation of the contents.

None of the test compounds, at any concentration, had significant effects on maternal mortality, pregnancy rate, numbers of corpora lutea or implantation sites, percentages of live and dead fetuses per litter, resorption frequency, fetal sex ratio, or the frequencies of external or internal malformations. Technical xylene and each of its isomers resulted in reduced maternal gestational weight gain at 1000 and 2000 ppm. All three xylene isomers, as well as the technical mixture, caused reduced fetal weights with exposure to 1000 and 2000 ppm. Fetal weights were also reduced at the 500 ppm concentration of o-xylene.

Increased frequencies of skeletal variations were seen with all three xylene isomers at 2000 ppm, but not with the technical mixture. Increased skeletal variations were also seen at 1000 ppm o-xylene. Combined variations (external, visceral, plus skeletal) were seen at increased frequencies with 2000 ppm of either p- or o-xylene, and with 1000 ppm o-xylene. Overall, while there were differences in the effective concentrations for developmental effects among xylene isomers, there did not appear to be evidence for strong, qualitative differences in potency.

Ungvary et al. (1980) exposed pregnant CFY rats to individual xylene isomers by inhalation at concentrations of 0, 35, 346, or 692 ppm. Animals were kept in the exposure chambers for 24 hours per day on GD 7-14.

Maternal mortality was seen only among 4/30 females exposed to the highest concentration of m-xylene (692 ppm). Gestational weight gain decreased with increasing dose for each xylene isomer, but only reached statistical significance at the high concentration of m-xylene.

Complete litter resorptions were seen only at 692 ppm p-xylene (7/20) and the same concentration of o-xylene (2/20). Implantation frequency showed a significant decrease only with exposure to 692 ppm m-xylene. Mean litter size and fetal loss expressed as a percentage of implantation sites were altered only at the highest concentration of p-xylene.

Mean fetal weights were significantly decreased at 692 ppm of all three isomers. For o-xylene, 346 ppm was also associated with reduced fetal weights. Exposure to either o-xylene or p-xylene had significant effects on mean placental weights, but there was not necessarily a clear relationship between specific response and concentration. Skeletal retardation was observed with both o- and p-xylene, at 692 ppm for the former and all three concentrations for the latter. The frequency of extra ribs was also increased with 692 ppm p-xylene. No other significant external, internal, or skeletal anomalies or malformations were observed with any concentration of any isomer.

Each of the three xylene isomers exerted some degree of maternal toxicity at the highest concentration of 692 ppm. All three isomers were also associated with retarded fetal development, as evidenced by decreased fetal weight and/or symptoms of skeletal retardation. Effective concentrations for these fetal effects varied among isomers. In decreasing order of potency, their effectiveness was p-, o-, and m-xylene. At the same time, increased incidences of extra ribs were seen with m- and p-xylene, but not with o-xylene. The authors proposed that differences among the isomers in metabolism, and/or maternal toxicity, could explain these differences.

Ungvary and Tatrai (1985) studied the effects of technical xylene and/or its individual isomers by inhalation in pregnant rats, mice, and rabbits. Test animals from each species were exposed for 24 hours per day throughout organogenesis. The concentrations used of technical xylene and/or each isomer, for each species, were as follows:

- CFY rats: technical xylene at 0, 58, 438, or 784 ppm
- CFLP mice:
  - Technical xylene at concentrations of 0, 500 or 1000 mg/m^3 (0, 115 or 231 ppm)
  - Individual xylene isomers at concentrations of 0 or 500 mg/m^3 (0 or 115 ppm)
- NZ rabbits: technical xylene or one of its isomers at 0, 115, or 231 ppm

Maternal mortality was seen in rats with the high concentration of technical xylene: 1/20 dams died at 784 ppm. Among rabbits, 1/8 and 3/10 dams died at 231 ppm p-xylene and technical xylene, respectively. Data on maternal gestational weight gain were reported only for rabbits, and no significant effect was observed at any concentration for technical xylene or any of its isomers. Maternal liver weights were found to be increased in rabbits exposed to the high concentration of technical xylene only.

Parameters of fetal viability in mice were not affected by technical xylene or any of its isomers. Rats showed no effect of technical xylene on the numbers of live fetuses per

group, but there was an increased percentage of dead or resorbed fetuses at the high concentration of 784 ppm. Rabbits showed the greatest sensitivity to effects of xylene on embryo-fetal mortality, as follows:

- 3/8 and 6/10 litters were totally aborted at 231 ppm p-xylene and technical xylene, respectively.
- 1/10, 4/8, and 1/10 litters were totally resorbed or dead at 115 ppm p-xylene, 231 ppm p-xylene, and 231 ppm technical xylene, respectively.
- The percentage of dead or resorbed fetuses, among total fetuses, was significantly increased at 115 ppm m-xylene ($p \leq 0.05$).

Effects of xylene on fetal weight were evidenced in rats at 784 ppm technical xylene, as an increased percentage of fetuses considered to be weight-retarded. Mice showed a significant increase in the percentage of weight-retarded fetuses at 231 ppm technical xylene, but not with other isomers. In rabbits, none of the three xylene isomers (o-, m-, or p-) affected mean fetal weight. Technical xylene at 115 ppm was associated with a significant decrease in the mean weight of female rabbit fetuses.

In rats exposed to technical xylene, increased percentages of fetuses were found to have evidence of skeletal retardation as compared to controls at 58, 438, and 784 ppm. At 784 ppm, increased percentages of rat fetuses were found to have minor anomalies, such as extra ribs. Mice exposed to 231 ppm technical xylene showed evidence of skeletal retardation, but neither xylene nor any of its isomers affected the percentages of minor anomalies such as extra ribs. Rabbit fetuses did not show changes in the frequencies of skeletal retardation or the presence of anomalies with exposure to technical xylene or any of the three isomers.

Overall, this study seem to show some differences between species in sensitivity to xylene and its isomers, as well as slight differences in effectiveness among different isomers. These differences appear to be more quantitative than qualitative in nature.

In a study designed to evaluate developmental neurotoxicity, Rosen et al. (1986) exposed groups of 25 pregnant Sprague-Dawley rats to 0, 3500, or 7000 mg/m^3 (0, 807.31, or 1614.62 ppm) p-xylene for six hours per day on GD 7-16. Maternal gestational weight gain was decreased in the high-concentration group, but there were no effects on the numbers of litters produced in each group. No effects of p-xylene exposure were found on litter size, birthweight, or subsequent pup growth. Neither tests of locomotor activity, nor the acoustic startle response showed any effect of treatment.

Ungvary et al. (1981) exposed CFY rats to 0 or 692 ppm p-xylene on GD 10 (24 hours continuously) or GD 9-10 (48 hours continuously). Animals were sacrificed on GD 11, and their uterine contents examined. Uterine weight divided by the number of embryos found in each uterus was significantly decreased following 48 hours exposure to 692 ppm p-xylene. As evidence of embryolethality was not found, the effect on weight appeared to be due to smaller, rather than fewer, embryos.

E.2.4. Oral Exposure Studies of Developmental Toxicity in Animals

One study looked at the effects of xylene given by the oral route of exposure (Marks et al., 1982). Timed-pregnant CD1 mice were given mixed xylene by gavage three times per day on each of GD 6-15. Daily doses during treatment were 0, 0.52, 1.03, 2.06, 2.58, 3.10, and 4.13 mg/kg-day.

None of the 15 pregnant dams exposed to the highest dose of 4.13 mg/kg-day survived until gestation day 18. Maternal mortality was 32% of 38 treated dams in the 3.10 dose group. Maternal gestational weight gain was significantly decreased at 3.10 mg/kg-day, and maternal liver weights were significantly increased at doses of 2.06 and 2.58 mg/kg-day. All surviving pregnant dams were sacrificed on GD 18, and their uterine contents examined using standard teratological techniques.

No effects of xylene were observed on implantation rate, mean live litter size, or sex ratio of viable fetuses. At the high dose of 3.10 mg/kg-day, 13/20 litters were completely resorbed, and the overall resorption frequency out of total implants was significantly increased.

Mean gravid uterine weights, as well as mean fetal weights, showed significant and dose-dependent reductions at the three highest doses of 2.06, 2.58, and 3.10 mg/kg-day. Morphological effects primarily consisted of cleft palate and a scattering of skeletal anomalies. The frequency of total malformations, expressed as a mean percentage, was significant at doses of 2.06, 2.58, and 3.10 mg/kg-day. Total malformations showed a significant trend to increase in frequency with increasing dose.

Overall, this study provides evidence for an effect of oral exposure to xylene on fetal weight and morphological development. These effects were seen at doses that were not associated with excessive maternal toxicity, as defined in the U.S. EPA Guidelines for Developmental Toxicity Risk Assessment (1991).

E.2.5. Dermal Exposure Studies of Developmental Toxicity in Animals

One study evaluated the neurotoxic effects of dermal exposure to xylene on pregnant Wistar rats and their offspring (Mirkova et al., 1979). Applied doses were 0, 100, 200, and 2000 mg/kg bw, daily throughout the pregnancy (GD 1-20). Pregnant dams were evaluated for open field behavioral activity on GD 18-20. At term, maternal and fetal brains were evaluated for biochemical endpoints.

Results from the open field test showed significantly reduced motor activity in pregnant females exposed to 2000 mg/kg xylene. The number of defecations while in the open field test was taken as an index of "emotionality," and was also significantly affected by exposure to the high dose of xylene. It is not clear from the paper whether this was an increase or decrease in the number of defecations, relative to controls. Nonetheless, these changes were taken as confirmation of xylene-induced neurotoxicity in the maternal animals.

According to the text of the paper, maternal brain cholinesterase (ChE) and cytochrome oxidase (CytO) activities were significantly decreased in a dose-dependent manner. The highest dose of xylene, 2000 mg/kg, was associated with increased glucose-6-phosphate dehydrogenase (G_6PDH) activity. The text also states that DNA concentrations and soluble protein content were reduced at 2000 and 200 mg/kg xylene.

Biochemical studies of fetal brains were stated to have found that ChE and CytO activities in fetal brains were inhibited at the 2000 and 200 mg/kg doses. At the same doses, fetal brain showed increases in the activities of MHD [undefined in the paper, but OEHHA believes this to be a typo for MDH, or malate dehydrogenase], isocitrate dehydrogenase, and G_6PDH.

Unfortunately, this study is poorly reported with regards to both methodology and results. Information on the methodology for application of xylene was not provided, nor were tabulated data. Taken at face value, however, this study does suggest that xylene can be absorbed by the dermal route and may measurably affect fetal tissues.

Table 7a. Animal Studies of Developmental Toxicity, Inhalation Route

Reference	Study design	Maternal toxicity	Developmental toxicity
Biodynamics (1983)	1-generation reproduction study; CD rats; N=10-40; 0, 60, 250, and 500 ppm; inhalation; daily, 6 hr/day; 131-days pre-mating, 20 day mating period, gestation and lactation 3 groups at 500 ppm: ♂ & ♀ treated ♀ only treated ♂ only treated On GD 21, 20 ♀s each from the 0 and 500 ppm groups (♂ & ♀ treated) sacrificed for teratology study Remaining ♀s delivered normally	No mortality in treated animals No clinical symptoms of toxicity No effect on body weight No gross pathology	<u>Teratology part of study:</u> ↓ Wt of ♀ fetuses at 500 ppm (♂ & ♀ treated) No effects on implantations, corpora lutea, resorptions, fetal viability, sex ratio, wt of ♂ fetuses, or frequencies of external, internal, or skeletal variations or malformations <u>Reproductive part of study:</u> No effect on pup survival to PND 4 ↑ Pup survival from PND 4 to PND 21 at 250 and 500 (♂ only treated) ppm ↓ Pup wts on PND 14, 21, and 49 at 500 ppm (♂ & ♀ treated) ↓ Ovary wts on PND 21, but not PND 49, at 250 and 500 ppm (♂ & ♀ treated)
Hass and Jakobsen (1993)	Rats, Wistar; 0, 200 ppm technical xylene; N = 36/group 6 hrs/day on GDs 4-20 2/3 litters evaluated at term, 1/3 for postnatal development and behavior	No clinical signs of maternal toxicity No effect on maternal body wt, food consumption, or uterine wts	<u>At term:</u> No significant effects on viability, fetal wt, or sex ratio; no↑ external or internal malformations; ↑ delayed ossification of the os maxilliare <u>Postnatal effects</u>: ↑ Birth wt. ♂ pups ↑ wts PND 28, both sexes Early pinna unfolding and eye opening ↓ Rotarod time, ♀ PND 22 and 23, ♂ PND 23
Hass et al. (1995)	Rats, Mol:WIST; 0, 500 ppm technical xylene; N = 13-15 6 hrs/day on GDs 7-20. PND 0 was defined as GD 22 Litters ≤ 6 pups were excluded from further evaluation.	No evidence for maternal toxicity, or for adverse impacts of exposure on gestation.	No significant effects on litter endpoints Delayed air-righting reflex No delays in other developmental landmarks Trend for exposed pups to show impairment in the Rotarod test, most marked in♀. On PND 26 impaired water maze performance by treated females

Table 7a. Animal Studies of Developmental Toxicity, Inhalation Route (continued)

Hass et al. (1997)	Rats, Mol:WIST; 0, 500 ppm technical xylene; N = 13-17 6 hrs/day on GDs 7-20. 1 ♀ pup/litter retained for testing in the Morris water maze at 12, 16, 28, 55 weeks postnatal	No evidence for maternal toxicity, or for embryo/fetal toxicity at birth, or effects on postnatal growth	Significant effects on water maze latency at 16 and 28 weeks. No effect on swim speed; effects on swim path lengths
Hudak and Ungvary (1978)	CFY rats; 0 or 1000 mg/m^3 (230 ppm); N = 20-28; 24 hrs/day, GD 9-14.	No effect on maternal mortality or maternal gestational weight gain	No effects on fetal loss, mean litter size, fetal weights, or placental weights ↑ Skeletal anomalies
Kükner et al. (1997)	Wistar rats; 0 or 11284 mg/m^3 (2600 ppm); 8 hr/day, 7 days/wk, GD 6-term; N = 5-7	No effect on mortality or gestational weight gain ↑ activity of liver AST, ALT, and arginase ↑ liver ALP level	Histopathological changes in liver tissues No evidence of effects on other organs; no external or internal gross anomalies; no histopathological changes in pancreas or kidney
Litton Bionetics (1978)	Rat; 0, 100, 400 ppm;6 hr/day, GD 6-15; N = 26	No effect on maternal mortality, gestational weight gain, or food consumption; no evidence of organ pathology	No effect on fetal viability or weight No external or visceral abnormalities reported No clear evidence of skeletal anomalies reported
Mirkova et al. (1983)	Developmental toxicity; Wistar rats; inhalation; 0, 10, 50, or 500 mg/m^3 (0, 2.31, 11.53, or 115.33 ppm); 6 hrs/day, 5 days/ week, GD 1-21	No mention of maternal mortality or weight gain	No change in gestation index, corpora lutea/litter, implantations/litter, live fetuses/litter, or resorptions as % of implantations ↑ Post implantation loss and autolyzed fetuses/implantations at 11.53 and 115.33 ppm ↓ Fetal wt at 11.53 and 115 ppm ↑ % total fetuses with hemorrhages at 11.53 and 115 ppm ↓ Postnatal growth ↑ AcP, AP, G_6PDH, LDH[4] in the lung tissues on PND 21
Rosen et al. (1986)	Sprague-Dawley rats; inhalation 0, 3500, 7000 mg/m^3 (0, 807.31, 1614.62 ppm) p-xylene; GD 7-16; N = 25 Dams gave birth normally	↓ maternal weight gain during treatment in the high-dose group	No effect on number of females giving birth /group or litter size No effect on birth wt or postnatal growth No effects on locomotor activity or acoustic startle response

[4] Acid phosphatase (AP), alkaline phosphatase (AcP), glucose-6-phosphate dehydrogenase (G_6PDH), and lactate dehydrogenase (LDH).

Table 7a. Animal Studies of Developmental Toxicity, Inhalation Route (continued)

Saillenfait et al. (2003)	Sprague-Dawley rats; inhalation, o-, m-, p-xylene, or technical xylene 0, 100, 500, 1000, or 2000 ppm; 6 hrs/day, GD 6-20; N = 20-26	No effect on maternal mortality ↓ gestational wt gain, 1000 and 2000 ppm, all compounds ↓ corrected maternal wt gain and feed consumption, 2000 ppm technical xylene, and 1000 and 2000 ppm of other compounds	<u>All isomers and technical mixture</u> No effect on pregnancy rate, number of corpora lutea, number of implantation sites, percentages of live and dead fetuses per litter, resorption frequency, fetal sex ratio, or external or internal malformations, ↓ Fetal wts at 1000 and 2000 ppm <u>o-xylene</u> ↓ Fetal wts at 500 ppm ↑ Skeletal variations at 1000 (as well as 2000) ppm ↑ Combined variations (external, visceral, skeletal) 1000 and 2000 ppm <u>p-xylene</u> ↑ Combined variations (external, visceral, skeletal) at 2000 ppm <u>o-, m-, and p-xylene</u> ↑ Skeletal variations with 2000 ppm of all three isomers
Ungvary et al. (1980)	CFY rats; inhalation, individual xylene isomers: 0, 150, 1500, or 3000 mg/m^3 (0, 35, 346, or 692 ppm); 24 hr/day, GD 7-14; N = 15-30	Maternal mortality: 4/30 at 692 ppm m-xylene ↓ Gestational wt gain at 692 ppm m-xylene ↓ Liver/body wt ratio at all concentrations of o-xylene, no clear dose effect	<u>o-xylene</u> Total resorption of 2/20 litters at 692 ppm ↓ Mean fetal wt at 346 and 692 ppm ↓ Mean placental weight at 35 ppm ↑ Mean placental weight at 346 and 692 ppm ↑ Skeletal retardation at 692 ppm <u>m-xylene</u> ↓ Implantation sites-dam at 692 ppm ↓ Mean fetal wt at 692 ppm ↑ Extra ribs at 692 ppm <u>p-xylene</u> Total resorption of 7/20 litters at 692 ppm p-xylene ↓ Mean litter size and fetal loss/implantation sites at 692 ppm ↓ Mean fetal wt at 692 ppm ↓ Mean placental weight at 35, 346, and 692 ppm ↑ Skeletal retardation at 35, 346, and 692 ppm ↑ Extra ribs at 692 ppm

Table 7a. Animal Studies of Developmental Toxicity, Inhalation Route (continued)

Ungvary et al. (1981)	CFY rats; inhalation, p-xylene: 0, 3000 mg/m^3 (0, 692 ppm); GD 10 or GD -10 (continuous 24 or 48 hrs); N = 8-14 Uterine contents evaluated on GD 11	No information provided	↓ Wt of uterine contents on GD 11 with 48 hrs exposure
Ungvary and Tatrai (1985)	CFY rats; 0, 250, 1900, or 3400 mg/m3 (0, 58, 438, or 784 ppm) ; 24 hrs/day, GD 7-15; N = 20-23 CFLP mice; xylene or single isomers; 0, 500, or 1000-1500 mg/m3 (0, 115, or 231-346 ppm); 3, 4-hr sessions/day, GD 6-15; N = 15-18 (115 controls) NZ rabbits; xylene or single isomers; 0, 500, or 1000 mg/m^3 (0, 115, or 231 ppm); 24 hrs/day, GD 7-20; N = 9-10 (60 controls)	Rats: 1/20 dams died at 784 ppm xylene Mice: no effects on maternal mortality Rabbits: 1/8 and 3/10 dams died at 231 ppm p-xylene and technical xylene, respectively; ↑ % maternal liver weight at 231 ppm technical xylene	<u>Rats</u> ↑ % dead or resorbed fetuses at 784 ppm, but no corresponding effect on the numbers of live fetuses per group ↑ % of fetuses with skeletal retardation at 58, 438, and 784 ppm ↑ % of weight retarded fetuses, and fetuses with minor anomalies. <u>Mice</u> ↑ % fetuses with weight retardation, and skeletal retardation at 115 ppm for all three individual isomers. Same effects only at 231 ppm for technical xylene <u>Rabbits</u> 3/8 and 6/10 litters aborted at 231 ppm p-xylene and technical xylene, respectively; 1/10, 4/8, and 1/10 litters totally resorbed or dead at 115 ppm p-xylene, 231 ppm p-xylene, and 231 ppm technical xylene, respectively ↑ % dead or resorbed fetuses at 115 ppm m-xylene ↓ wt of ♀ fetuses at 115 ppm technical xylene

Table 7b. Animal Study of Developmental Toxicity, Oral Route

Reference	Study design	Maternal toxicity	Developmental toxicity
Marks et al. (1982)	CD-1 mice; 0, 0.52, 1.03, 2.06, 2.58, 3.10, 4.13 mg/kg-day; N = 23-66; GD 6-15.	Maternal mortality: 100% at 4.13 mg/kg-day, 32% at 3.10 mg/kg-day. ↓ gestational weight gain at 3.10 mg/kg-day ↑ liver weights at 2.06 and 2.58 mg/kg-day ↓ uterine weights at 2.06, 2.58, and 3.10 mg/kg-day	At 3.10 mg/kg-day, 13/20 litters completely resorbed; remaining litters, resorption frequency = 62.3% ↓ Fetal weight at 2.06, 2.58, and 3.10 mg/kg-day ↑ total malformations, at 2.06, 2.58, and 3.10 mg/kg-day; dose-response trend

Table 7c. Animal Study of Developmental Toxicity, Dermal Route

Reference	Study design	Maternal toxicity	Developmental toxicity
Mirkova et al. (1979)	Wistar rats, N = 80; dermal application of 0, 100, 200, and 2000 mg/kg bw-day, GD 1-20 Dams evaluated for open field activity on GD 18-20. At term, maternal and fetal brains evaluated for enzyme activities, DNA concentration, and protein content	↓ motor activity in pregnant females exposed to 2000 mg/kg xylene ↓maternal brain ChE[5] and CytO activities in a dose-dependent manner. ↑ G_6PDH activity, 2000 mg/kg, was associated with. ↓ DNA and protein at 2000 and 200 mg/kg xylene.	↓ChE and CytO activities in fetal brains at 2000 and 200 mg/kg, ↑ MHD, i-CDH, and G_6PDH activity.

E.2.6. Related Information of Relevance to the Potential Developmental Toxicity of Xylene

As part of a classic study of the teratogenic potential of fat solvents, Kucera (1968) exposed chick embryos to xylene vapors. A total of 317 chick embryos were divided into three age groups, according to classic Hamburger and Hamilton (HH) terminology. The embryos were exposed to xylene-containing air for 60, 120, 180, or 240 minutes. Exposure occurred between the developmental stages of HH-3 to HH-14 (approximately corresponding to gestation days 16-24 in humans).

Brief exposure to xylene of the youngest group (HH-3 to HH-5) resulted in significantly increased mortality and malformations. Malformation frequency increased with increasing length of exposure, and decreased with increasing embryo age. The highest malformation frequency was seen after an exposure of 180 minutes. The highest mortality rate was seen with an exposure time of 240 minutes to chick embryos at developmental stages lower than HH-10. Nearly half of the malformed chick embryos were classified as "rumpless," a condition the author described as analogous to caudal regression in humans.

[5] Brain cholinesterase (ChE), cytochrome oxidase (CytO), glucose-6-phosphate dehydrogenase (G_6PDH), MHD [undefined in the paper, but OEHHA believes this to be a typographical error for MDH, or malate dehydrogenase], isocitrate dehydrogenase (i-CDH)

Table 8. Malformations and Mortality in Chick Embryos Exposed to Xylene

Exposure	HH 3-5		HH 6-10		HH 11-14		HH 3-14	
Endpoint	M/N	D/N	M/N	D/N	M/N	D/N	M%	D%
60 min.	4/12	5/12	5/18	7/18	4/59	11/59	14.6%	25.8%
120 min.	11/22	9/22	12/39	10/39	4/27	0/27	30.7%	21.6%
180 min.	11/18	3/18	12/28	4/28	3/26	0/26	36.1%	9.7%
240 min.	2/18	14/18	7/24	9/24	4/26	0/26	19.1%	33.8%
Total	28/70	31/70	36/109	30/109	15/138	11/138	24.9%	22.8%
Total %	40%	44%	33%	28%	11%	8%	—	—

M/N — number malformed/total
D/N — number dead/total

The author concluded that the data support xylene as a teratogen interfering with normal development of exposed chick embryos. There does not appear to have been any statistical analysis of these data.

As noted above in Section E.1.2, the paper also reported on nine cases of sacrococcygeal agenesis, or caudal regression, among infants born in Czechoslovakia between the years 1959 and 1966.

E.3. Integrative Evaluation for Developmental Toxicity

Nine epidemiologic studies examined the effect of xylene exposure on risk of adverse developmental outcomes (Axelsson et al., 1984; Ghosh et al., 2012; Lehman et al., 2002; Lindbohm et al., 1990; Lupo et al., 2011; Swan et al., 1995; Taskinen et al., 1989; Taskinen et al., 1994; Windham et al., 1991). Studies aimed to examine effects of groups of related chemicals or work in particular occupational settings or occupations. Exposure assessment was based on self-reported occupational exposure to xylene or occupational setting (Axelsson et al., 1984; Lindbohm et al., 1990; Lupo et al., 2011; Taskinen et al., 1989; Taskinen et al., 1994; Windham et al., 1991), estimated residential ambient BTEX levels (Lupo et al., 2011), measured outdoor ambient BTEX levels (Ghosh et al., 2012), or measured residential VOCs (Lehman et al., 2002). One study examined paternal exposure (Taskinen et al., 1989). Exposure to xylene was typically highly correlated with other exposures, and exposure to xylene alone was uncommon. As a result, the potential effects of xylene were difficult to distinguish from those of other exposures.

Fifteen studies were identified as having information on the developmental toxicity of technical or mixed xylene and/or its individual isomers in animals (Biodynamics, 1983; Hass et al., 1995; Hass et al., 1997; Hass and Jakobsen, 1993; Hudak and Ungvary, 1978; Kükner et al., 1997; Litton Bionetics, 1978; Marks et al., 1982; Mirkova et al., 1979; Mirkova et al., 1983; Rosen et al., 1986; Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary et al., 1981; Ungvary and Tatrai, 1985). An additional study contains related information on the effects of exposing chick embryos to xylene vapors (Kucera, 1968).

Nine of the 15 studies provided information on endpoints of developmental toxicity for technical xylene by the inhalation route of exposure (Biodynamics, 1983; Hass and Jakobsen, 1993; Hass et al., 1995; Hass et al., 1997; Kükner et al., 1997; Litton Bionetics, 1978; Mirkova et al., 1983; Saillenfait et al., 2003; Ungvary and Tatrai, 1985). Some of these studies also provided information on the developmental toxicity of individual xylene isomers, or included data on postnatal developmental effects. All of these studies used rats as the test species except for Ungvary and Tatrai (1985), which compared the effects observed in rats, mice, and rabbits.

Three studies compared the developmental effects of inhaled technical xylene with each of the individual xylene isomers (Saillenfait et al., 2003; Ungvary et al., 1980; Ungvary and Tatrai, 1985). Two additional studies evaluated the developmental effects of p-xylene only (Rosen et al., 1986; Ungvary et al., 1981).

One study looked at the effects of xylene given to mice by the oral route of exposure (Marks et al., 1982), and one study evaluated the neurotoxic effects of dermal exposure to xylene on pregnant Wistar rats and their offspring (Mirkova et al., 1979).

Offspring viability

Six epidemiologic studies provide limited evidence for exposure to xylene as a risk factor for SAB or miscarriage, including one study that examined paternal exposure (Axelsson et al., 1984; Lindbohm et al., 1990; Swan et al., 1995; Taskinen et al., 1989; Taskinen et al., 1994; Windham et al., 1991). In the paternal exposure study by Taskinen et al. (1989), the adjusted ORs of 1.7 and 1.6 for intermediate and high/frequent paternal exposure to xylene, respectively, indicate excess risk of SAB, but were not statistically significant. Additionally, only 11 subjects were exposed to xylene without other chemicals, and the recall period was up to 14 yrs. Of five studies examining risk of SAB associated with maternal occupational exposure to xylene, two found significantly increased risk of SAB among female laboratory workers (Taskinen et al., 1994) and women working in the semiconductor industry (Swan et al., 1995). As with other human studies of xylene exposure, these studies shared the limitation of simultaneous exposure to other compounds associated with SAB and few subjects exposed to xylene alone. Three other studies reported either non-significant associations between maternal xylene exposure and risk of SAB (Lindbohm et al., 1990 and Windham et al., 1991), or no association (Axelsson et al., 1984).

The only animal study to report adverse effects of inhaled xylene on offspring viability was Ungvary and Tatrai (1985). Rats showed a significant increase in the percentage of dead or resorbed fetuses at the highest concentration of 784 ppm, but there was no corresponding effect on the numbers of live fetuses in this group. No effects on viability were observed on mice in this study, but rabbits appeared to be more sensitive. At the highest concentration of 231 ppm technical xylene: 3/10 pregnant rabbits died, six of the remaining litters completely aborted, and the final litter consisted entirely of resorbed fetuses. Viability of does and fetuses was unaffected at the lower dose of 115 ppm.

At a dose of 3.10 mg/kg-day, xylene given by the oral gavage route of exposure to pregnant mice caused an increase in resorption frequency, as well as complete resorption of 13/20 litters (Marks et al., 1982). Maternal mortality was 32% at this dose, a level greater than the 10% maternal mortality defined in the U.S. EPA Risk Assessment Guidelines for Developmental Toxicity (1991) as "minimal." Therefore, the offspring mortality data from this study are difficult to interpret.

Fetal growth

Four epidemiologic studies examined birth weight or fetal growth (Axelsson et al., 1984; Ghosh et al., 2012; Taskinen et al., 1994; Windham et al., 1991). Two reported results for xylene (Ghosh et al., 2012; Taskinen et al., 1994), and findings were mixed. Ghosh et al. reported increased odds of term LBW associated with increases in ambient (m+p)-xylene and o-xylene. However, Taskinen et al. reported maternal exposure to xylene up to two days per week was associated with increased birth weight, though no data or statistics were provided.

Fetal or birth weights were found to be affected by prenatal exposure to technical xylene by the inhalation route in several animal studies, but not all. Results for individual xylene isomers generally, but not always, showed effects on offspring weight.

Hass and Jakobsen (1993) found no effects on fetal weight in rats following gestational exposure to xylene at 200 ppm. However, among those animals that were allowed to carry their litters to term, birth weights for treated male pups were significantly increased over controls. Birth weights were not affected by exposure to 500 ppm xylene in either of two rat studies by Hass et al. (1995, 1997). Hudak and Ungvary (1978) found no effects on fetal weights in rats following exposure to 230 ppm xylene. Litton Bionetics (1978) found no effects on fetal weights in the rat fetuses exposed to 100 or 400 ppm xylene.

In contrast, Biodynamics (1983) found a significant decrease in the weights of female rat fetuses at a xylene concentration of 500 ppm, when both paternal and maternal animals were exposed. Mirkova (1983) reported decreased fetal weights in rats with inhalation exposure to 11.53 or 115 ppm xylene.

Saillenfait et al. (2003) found that all three xylene isomers, as well as the technical mixture, caused reduced fetal weights in rats with exposure to 1000 and 2000 ppm. Weights of fetal rats were also reduced at the 500 ppm concentration of o-xylene. Ungvary et al. (1980) found mean fetal weights for rats were significantly decreased at 692 ppm of all three isomers. For o-xylene, 346 ppm was also associated with reduced fetal weights.

Ungvary and Tatrai (1985) found increased percentages of fetuses defined as weight-retarded at the highest concentrations of technical xylene tested in rats and mice (784 ppm and 231 ppm, respectively). In that same study, technical xylene at 115 ppm was associated with a significant decrease in the mean weight of female rabbit fetuses. None of the individual xylene isomers affected fetal weights in either mice or rabbits.

In the one oral study of xylene-induced developmental toxicity in mice, mean fetal weights were significantly reduced at doses of 2.06, 2.58, and 3.10 mg/kg-day, in a dose-dependent manner (Marks et al., 1982). The one dermal study provided no information relevant to fetal growth (Mirkova et al., 1979).

Malformations

Four epidemiologic studies examined exposure to xylene as a risk factor for malformations (Axelsson et al., 1984; Lupo et al., 2011; Taskinen et al., 1989; Taskinen et al., 1994). A study of maternal exposure to BTEX as a risk factor for spina bifida and anencephaly found that all ORs for xylene exposure above the lowest level exceeded unity, suggesting an effect of xylene, but no OR was statistically significant, and all of the BTEX compounds were highly correlated (Lupo et al., 2011). Other studies were likely too small to observe an effect of xylene and had other challenges in methods, such as multiple exposures, long recall, and heterogeneous outcomes. One study examined paternal exposure to xylene and other organic solvents and reported a non-significant association with any malformation (Taskinen et al., 1989). This study also included only 25 malformation cases, few exposed cases (including malformations and other outcomes), and a long recall period for exposure assessment. Two other studies reported no association between xylene exposure and malformations (Taskinen et al., 1994 and Axelsson et al., 1984).

Among the animal studies of technical xylene exposure by the inhalation route, none reported significant increases in the frequencies of external or internal, soft-tissue malformations or anomalies. Several studies that evaluated fetal skeletal development, reported effects with technical xylene exposure; other studies reported no skeletal effects. Results for inhalation studies of individual xylene isomers also have not shown evidence for external or internal soft-tissue abnormalities, and had mixed results for skeletal effects. The one mouse oral study found no skeletal effects, but did show a treatment-related increase in overall malformation frequency.

Biodynamics (1983) reported no effect of xylene exposure on fetal skeletal development at concentrations as high as 500 ppm (the highest dose tested). Litton Bionetics (1978) reported no clear evidence for a xylene-induced increase in skeletal anomalies following prenatal exposure to concentrations of 100 or 400 ppm. Saillenfait et al. (2003) reported no increase in the frequency of skeletal anomalies with prenatal exposure to technical xylene at concentrations up to and including 2000 ppm.

Hass and Jakobsen (1993) reported a significant increase in the frequency of delayed ossification of the *os maxilliare* at the test concentration of 200 ppm xylene. Hudak and Ungvary (1978) reported an increase in skeletal anomalies with exposure to 230 ppm xylene. In rats exposed to technical xylene, Ungvary and Tatrai (1985) found increased percentages of fetuses to have evidence of skeletal retardation at 58, 438, and 784 ppm. At 784 ppm, increased percentages of rat fetuses were found to have minor skeletal anomalies, such as extra ribs. Mice exposed to 231 ppm technical xylene showed evidence of skeletal retardation, but neither xylene nor any of its isomers

affected the percentages of minor anomalies such as extra ribs. Rabbit fetuses did not show changes in the frequencies of skeletal retardation or the presence of anomalies with exposure to technical xylene or any of the three isomers.

Saillenfait et al. (2003) reported increased skeletal variations at 2000 ppm o-xylene, p-xylene, or m-xylene, but not with the technical mixture. With exposure to o-xylene at concentrations of 1000 or 2000 ppm, significant increases were seen in the number of fetuses with any variations (external, visceral, and skeletal combined). Increased combined malformations and variations were also seen for p-xylene at 2000 ppm, but not with m-xylene or technical xylene at either concentration.

Ungvary et al. (1980) observed skeletal retardation following prenatal exposure to 692 ppm o-xylene, and with p-xylene at 35, 346, and 692 ppm. The frequency of extra ribs was also increased with 692 ppm p-xylene. No other significant external, internal, or skeletal anomalies or malformations were observed with any concentration of any isomer.

A study of oral exposure to technical xylene in mice reported no increase in the frequency of skeletal abnormalities even at the high dose of 4.13 mg/kg-day (Marks et al., 1982). This study reported significant increases in the frequencies of total malformations at doses of 2.06, 2.58, and 3.10 mg/kg-day, with an apparent dose-response relationship. Cleft palate was the most common malformation, while bilateral open eye, exencephaly, and skeletal findings such as fused or missing vertebral arches and ribs.

Developmental neurotoxicity
No studies of developmental neurotoxicity of xylene in humans were identified.

Four animal studies looked specifically at the developmental neurotoxicity of prenatal exposure to xylene. Only one of these four considered p-xylene in isolation, rather than the technical xylene mixture.

Hass and Jakobsen (1993) found reduced Rotarod times for female rat pups prenatally exposed to technical xylene at a concentration of 200 ppm. For males, Rotarod time was significantly reduced only on the second of three test days. The authors concluded that impairment of Rotarod performance, which was mainly significant in females, could be interpreted as an indicator of impaired motor ability in xylene-treated animals.

Hass et al. (1995) found a significant delay in acquisition of the air-righting reflex in rat pups prenatally exposed to technical xylene at 500 ppm. As other developmental landmarks were unaffected by treatment, this finding was taken by the study authors to suggest damage to neural processes required for air righting, such as vestibular function, rather than a more general developmental delay. The Rotarod test did not reveal a significant effect of treatment, but a trend toward impaired performance was taken to indicate potential impairment of motor coordination and balancing abilities in

exposed female offspring. No effects of treatment were seen on an Open Field test, but some changes were seen on the Morris water maze test. In particular, exposed female offspring showed a significant increase in swimming time, but no difference in swimming speed relative to controls.

Hass et al. (1997) followed up on their previous results with an additional study of female rats exposed *in utero* to 500 ppm xylene by inhalation. Increased latencies in the Morris water maze were observed in these animals when tested at postnatal ages of 16, 28, and 55 weeks. These differences did not appear to be related to swim speed or other motor difficulties with swimming, but rather with increased swim path lengths.

The final behavioral study used pregnant rats exposed to 0, 807.31, or 1614.62 ppm p-xylene for six hours/day on GD 7-16 (Rosen et al., 1986). Neither tests of locomotor activity, nor the acoustic startle response test showed any effect of treatment.

Other developmental outcomes
One study in humans examined the effect of exposure to VOCs on alterations of the cytokine secretion profile of cord blood T-cells (Lehman et al., 2002). In multivariate analyses, xylenes were not associated with cord blood cytokine-producing T-cells.

References

Aguilar-Garduno, C., M. Lacasana, et al. (2010). "Parental occupational exposure to organic solvents and anencephaly in Mexico." Occupational and Environmental Medicine **67**(1): 32-37.

Aguilera, I., R. Garcia-Esteban, et al. (2010). "Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort." Environmental health perspectives **118**(5): 705-711.

Aguilera, I., M. Guxens, et al. (2009). "Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort." Environmental health perspectives **117**(8): 1322-1327.

ATSDR (2007). "Toxicological profile for xylene " US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Axelsson, G. L., C; Rylander, R (1984). "Exposure to solvents and outcome of pregnancy in university laboratory employees." British Journal of Industrial Medicine **41**(3): 305-312.

Biodynamics, Inc., (1983). "Parental and fetal reproduction toxicity study in rats with mixed xylenes." Submitted to the U.S. Environmental Protection Agency under TSCA Section FYI. FYI00002091.

California Air Resources Board. (2012a). "Annual Statewide Toxics Summary: meta/para-Xylene." Retrieved 8/17/12, from http://www.arb.ca.gov/adam/toxics/statepages/mpxylstate.html.

California Air Resources Board. (2012b). "Annual Statewide Toxics Summary: ortho-Xylene." Retrieved 8/17/12, from http://www.arb.ca.gov/adam/toxics/statepages/oxylstate.html.

Chen, H. S., Ling; Wang, Xinru; Wang, Shoulin; et al., (2000). "Effect of exposure to low concentration of benzene and its analogues on luteal function of female workers." Journal of Hygiene Research **29**(6): 351-353.

Chevrier, C., B. Dananche, et al. (2006). "Occupational exposure to organic solvent mixtures during pregnancy and the risk of non-syndromic oral clefts." Occupational and Environmental Medicine **63**(9): 617-623.

Cho, S. I., A. I. Damokosh, et al. (2001). "Effects of exposure to organic solvents an menstrual cycle length." Journal of Occupational and Environmental Medicine **43**(6): 567-575.

Chung, W. G., I. J. Yu, et al. (1999). "Decreased formation of ethoxyacetic acid from ethylene glycol monoethyl ether and reduced atrophy of testes in male rats upon combined administration with toluene and xylene." Toxicology letters **104**(1-2): 143-150.

De Celis, R., A. Feria-Velasco, et al. (2000). "Semen quality of workers occupationally exposed to hydrocarbons." Fertil Steril **73**(2): 221-228.

Garlantezec, R., C. Monfort, et al. (2009). "Maternal occupational exposure to solvents and congenital malformations: a prospective study in the general population." Occupational and Environmental Medicine **66**(7): 456-463.

Ghantous, H. and B. R. Danielsson (1986). "Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice." Biological research in pregnancy and perinatology **7**(3): 98-105.

Ghosh, J. K. C., M. Wilhelm, et al. (2012). "Assessing the Influence of Traffic-related Air Pollution on Risk of Term Low Birth Weight on the Basis of Land-Use-based Regression Models and Measures of Air Toxics." American Journal of Epidemiology **175**(12): 1262-1274.

Ha, E., S. I. Cho, et al. (2002). "Parental exposure to organic solvents and reduced birth weight." Archives of Environmental Health **57**(3): 207-214.

Hass, U. and B. M. Jakobsen (1993). "Prenatal toxicity of xylene inhalation in the rat: a teratogenicity and postnatal study." Pharmacology & toxicology **73**(1): 20-23.

Hass, U., S. P. Lund, et al. (1997). "Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats." Neurotoxicology **18**(2): 547-551.

Hass, U., S. P. Lund, et al. (1995). "Effects of prenatal exposure to xylene on postnatal development and behavior in rats." Neurotoxicology and teratology **17**(3): 341-349.

Holmberg, P. C. and M. Nurminen (1980). "Congenital defects of the central nervous system and occupational factors during pregnancy. A case-referent study." Am J Ind Med **1**: 167-176.

Hudak, A. and G. Ungvary (1978). "Embryotoxic effects of benzene and its methyl derivatives:toluene,xylene." Toxicology **11**: 55-63.

IARC (1989). "Xylene." IARC Monogr Eval Carcinog Risks Hum **47**: 125-156.

Khattak, S., G. K-Moghtader, et al. (1999). "Pregnancy Outcome Following Gestational Exposure to Organic Solvents." JAMA: The Journal of the American Medical Association **281**(12): 1106-1109.

Kucera, J. (1968). "Exposure to fat solvents: a possible cause of sacral agenesis in man." J Pediatr **72**: 857-859.

Kükner, A. C., L; Ozan, E; Gökçimen, A; Ozan, S; Doğrul, M (1997). "The effect of xylene inhalation on the rat liver." Acta physiologica Hungarica **85**(3): 231-241.

Laslo-Baker, D., M. Barrera, et al. (2004). "Child neurodevelopmental outcome and maternal occupational exposure to solvents." Arch Pediatr Adolesc Med **158**(10): 956-961.

Lehmann, I., A. Thoelke, et al. (2002). "The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells." Environ. Toxicol. **17**(3): 203-210.

Lemasters, G. K., D. M. Olsen, et al. (1999). "Male reproductive effects of solvent and fuel exposure during aircraft maintenance." Reproductive toxicology (Elmsford, N.Y.) **13**(3): 155-166.

Lindbohm, M. L., H. Taskinen, et al. (1990). "Spontaneous abortions among women exposed to organic solvents." Am J Ind Med **17**(4): 449-463.

Litton Bionetics (1978). Teratology study in rats xylene (final report). Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. OTS0205935.

Lupo, P. J., E. Symanski, et al. (2011). "Maternal exposure to ambient levels of benzene and neural tube defects among offspring: Texas, 1999-2004." Environmental health perspectives **119**(3): 397-402.

Magnusson, L. L., L. Bodin, et al. (2006). "Adverse pregnancy outcomes in offspring of fathers working in biomedical research laboratories." American Journal of Industrial Medicine **49**(6): 468-473.

Marks, T. A., T. A. Ledoux, et al. (1982). "Teratogenicity of a commercial xylene mixture in the mouse." Journal of toxicology and environmental health **9**(1): 97-105.

Merck, Ed. (1989). The Merck Index. Rahway, N.J., Merck & Co., Inc.

Mirkova, E., C. Zaikov, et al. (1983). "Prenatal toxicity of xylene." Journal of hygiene, epidemiology, microbiology, and immunology **27**(3): 337-343.

Mirkova E, H. L., Vassileva L, et al. (1979). "Xylene neurotoxicity in pregnant rats and fetuses." Act Nerv Super (Praha) **21**: 265-268.

Nylen P, E. T., Eriksdotter-Nilsson M, et al. (1989). "Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene." Arch Toxicol Suppl **63**: 296-307.

OEHHA (1997). Public Health Goal for Xylene in Drinking Water.

OEHHA (1999). "Determination of Acute Reference Exposure Levels for Airborne Toxicants: Appendix D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines " http://oehha.ca.gov/air/acute_rels/pdf/xylenesA.pdf.

OEHHA (2008). All OEHHA Acute, 8-hour and Chronic Reference Exposure Levels (chRELs) as on December 18, 2008.

Reutman, S. R., G. K. LeMasters, et al. (2002). "Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures." Environmental health perspectives **110**(8): 805-811.

Rosen, M. B., K. M. Crofton, et al. (1986). "Postnatal evaluation of prenatal exposure to p-xylene in the rat." Toxicology letters **34**(2-3): 223-229.

Saillenfait, A. M., F. Gallissot, et al. (2003). "Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure." Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association **41**(3): 415-429.

Sallmén, M., M.-L. Lindbohm, et al. (1998). "Time to pregnancy among the wives of men exposed to organic solvents." Occup. Environ. Med. **55**(1): 24-30.

Sallmén, M., M.-L. Lindbohm, et al. (1995). "Reduced fertility among women exposed to organic solvents." American Journal of Industrial Medicine **27**(5): 699-713.

South Coast Air Quality Management District (2008). Multiple Air Toxics Exposure Study in the South Coast Air Basin; MATES III, Final Report, South Coast Air Quality Management District, Diamond Bar, CA 91765.

Swan, S. H., J. J. Beaumont, et al. (1995). "Historical Cohort Study of Spontaneous Abortion Among Fabrication Workers in the Semiconductor Health Study – Agent-Level Analysis." American Journal of Industrial Medicine **28**(6): 751-769.

Taskinen, H., A. Anttila, et al. (1989). "Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents." Scand. J. Work, Environ. Health **15**(5): 345-352.

Taskinen, H., P. Kyyronen, et al. (1994). "Laboratory work and pregnancy outcome." Journal of occupational medicine. : official publication of the Industrial Medical Association **36**(3): 311-319.

Testud, F., A. D'Amico, et al. (2010). "Pregnancy outcome after risk assessment of occupational exposure to organic solvents: A prospective cohort study." Reproductive Toxicology **30**(3): 409-413.

Ungvary, G. and E. Tatrai (1985). "On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits." Arch Toxicol Suppl **8**: 425-430.

Ungvary, G., E. Tatrai, et al. (1980). "Studies on the embryotoxic effects of ortho-,meta- and para-xylene." Toxicology **18**: 61-74.

Ungvary, G., B. Varga, et al. (1981). "Study on the role of maternal sex steroid production and metabolism in the embryotoxicity of para-xylene." Toxicology **19**: 263-268.

U.S. EPA (2003a). "Toxicological Review of Xylenes (CAS No. 1330-20-7)."

U.S. EPA. (2003b (last revision)). "Integrated Risk Information System (IRIS): Xylenes (CASRN 1330-20-7) ", from http://www.epa.gov/IRIS/subst/0270.htm.

U.S. EPA (2010). TRI Explorer: TRI On-site and Off-site Reported Disposed of or Otherwise Released (in pounds), for All industries, for XYLENE (MIXED ISOMERS) chemical, California, 2010.

Wang, F. (1994). "Effects of benzene, toluene and xylene on female reproductive function." Gongye Weisheng Yu Zhiyebing **20**(2): 117-119.

Washington, W. J., R. C. Murthy, et al. (1983). "Induction of morphologically abnormal sperm in rats exposed to O-Xylene." Archives of andrology **11**(3): 233-237.

Wennborg, H., L. Bodin, et al. (2001). "Solvent use and time to pregnancy among female personnel in biomedical laboratories in Sweden." Occupational and Environmental Medicine **58**(4): 225-231.

Windham, G. C., D. Shusterman, et al. (1991). "Exposure to organic solvents and adverse pregnancy outcome." Am. J. Ind. Med. **20**(2): 241-259.

Windham, G. C., L. Zhang, et al. (2006). "Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay area." Environ. Health Perspect. **114**(9): 1438-1444.

Xiao, G., G. Pan, et al. (1999). "Effect of benzene, toluene, xylene on the semen quality of exposed workers." Chin. Med. J. (Beijing, Engl. Ed.) **112**(8): 709-712.

Xiao, G. B., C. B. Pan, et al. (2001). "Effect of Benzene, Toluene, Xylene on the Semen Quality and the Function of Accessory Gonad of Exposed Workers." Industrial Health **39**(2): 206-210.

Yamada, K. (1993). "Influence of lacquer thinner and some organic solvents on reproductive and accessory reproductive organs in the male rat." Biological & pharmaceutical bulletin **16**(4): 425-427.

Yang, S. (1997). "Epidemiological survey of the benzene series compounds on female reproductive function and offspring development." Gongye Weisheng Yu Zhiyebing **23**(4): 228-229.

Appendix A. Summaries of Epidemiologic Studies

In this appendix, the epidemiological studies discussed in the main document are summarized. The study summaries provided here are arranged in alphabetical order by the first author's last name and year of publication.

Axelsson et al., 1984 96

Cho et al., 2001 96

Ghosh et al., 2012 98

Lehman et al., 2002 100

Lindbohm et al., 1990 101

Lupo et al., 2011 102

Sallmén et al., 1995 104

Sallmén et al., 1998 106

Swan et al., 1995 108

Taskinen et al., 1989 110

Taskinen et al., 1994. 114

Windham et al., 1991 116

Xiao et al., 1999 and Xiao et al., 2001 117

Axelsson et al., 1984. Exposure to solvents and outcomes of pregnancy in university laboratory employees

The purpose of this cohort study was to study the relationship between laboratory work, particularly exposure to solvents, and pregnancy outcome (miscarriage, malformations, birth weight). Women who had worked at the University of Gothenburg were identified through computerized payrolls; those born in or after 1935 and who had been engaged in laboratory work (based on 39 job titles) between 1968 and 1979 were selected. Of the 811 female laboratory workers, 782 were still living and resident in Sweden, 745 (95%) responded to the questionnaire, and 556 reported having been pregnant. These women were asked to complete a mailed questionnaire concerning pregnancy outcomes (including induced abortions, ectopic pregnancies, and miscarriages), occupation during pregnancy, occupational exposure to solvents, smoking, use of medicines, and diseases during pregnancy. Xylene was one of seven solvents specifically named in the questionnaire. Additional information, such as medical data on the mother and child, marital status, and place of residence, was obtained from the Swedish Medical Birth Register. Information on malformations was obtained from the Swedish Register of Congenital Malformations.

Possible confounding factors, such as pregnancy number, age, year of pregnancy, previous miscarriage, and shift work, were evaluated. For miscarriage analyses, pregnancies during shift work were excluded, and the data were stratified by pregnancy number and maternal age. The miscarriage rate was defined as (miscarriages) ÷ (miscarriages + births + induced abortions). The authors observed no differences in miscarriage rates with respect to frequency of exposure to solvents, nor were there differences by the type of solvent, including xylene. There were 20 miscarriages among 194 women exposed to xylene during the first trimester, for a rate of 10.3%. No other results for xylene were reported.

The prevalence of malformations among women who were exposed to solvents (3.7%) was similar to that among unexposed women (4.2%). Various minor and major malformations were reported.

For the relationship between birth weight and exposure to solvents during the second and third trimester, the authors reported a correlation coefficient of 0.028 ($p=0.379$).

Comment
Length of recall period is not reported, but was at least five years for women who worked in laboratories in 1968 (the birth register started in 1973).

Cho et al., 2001. Effects of Exposure to Organic Solvents on Menstrual Cycle Length

The purpose of this cross-sectional study was to examine whether there is an association between low-level exposure to organic solvents and menstrual patterns in women employed in a petrochemical industry in Beijing, China. The major occupational exposures included aromatic solvents such as benzene, toluene, styrene, and xylene.

From 1994-1998, the authors recruited 20-40 year old nonparous female employees from BYPC, a large governmental industrial park of 17 different but well-integrated petroleum and chemical processing plants. These women were either newlyweds who had visited a district health center for a mandated marriage health examination (MHE) or married women who had visited a family planning office to obtain childbirth permission (CBP). Other eligibility criteria included no previous marriages, no previous clinical pregnancies, and no diagnosed gynecologic or endocrine diseases. All eligible workers in the MHE group were enrolled, whereas in the CBP group, only workers from nine production facilities were recruited. The nine plants were selected on the basis of feasibility of subsequent follow-up. The participation rate was 95% among the eligible women and the final sample size was 1,408.

Cho et al. had previously conducted research on exposure assessment and developed a database for evaluation of exposures in the various workshops at BYPC. Based on this previous work, an industrial hygienist classified each workshop as either exposed or unexposed to benzene, toluene, styrene, and/or xylene. In addition, women reported handling of specific chemicals on a checklist in the baseline questionnaire. The authors report that because BYPC is a modern industry, exposures are very low, with average levels of benzene well below the limit recommended by the National Institute for Occupational Safety and Health, and xylene, toluene, and styrene levels below 1 ppm.

Menstrual patterns in the year before enrollment were assessed in an interview at baseline. Information included average cycle length, longest and shortest cycle length, duration of bleeding, perceived irregularity, intermenstrual spotting, and perimenstrual symptoms. Oligomenorrhea was defined as an average cycle length greater than 35 days and included amenorrhea, polymenorrhea was defined as an average cycle length of less than 21 days, and menorrhagia was defined as an average bleeding period of greater than seven days.

The authors collected detailed information on the following potential confounders: age, weight, height, date of marriage, current and past contraceptive use, parity, history of active and passive smoking, indoor coal combustion and cooking oil fumes, alcohol use, diet, use of herbal medicines, heavy lifting, body position during work, rotating shift work, perceived work stress, and physical activities outside the workplace.

Multiple logistic regression was used to estimate the effect of solvent exposures with adjustment for covariates. Of the 284 newlywed women exposed to xylene, all were also exposed benzene and toluene, and 196 were exposed to benzene, toluene, and styrene. Among these xylene-exposed women, 14.1% had oligomenorrhea, whereas the prevalence of oligomenorrhea among women who had no solvent exposure was 8.5%. The OR for oligomenorrhea and exposure to xylene (with other solvents) was 1.63 (1.04-2.53), adjusted for age, body mass index, enrollment cohort, passive smoking, and exposure to chemicals other than aromatic solvents. The adjusted OR for oligomenorrhea and exposure to any aromatic solvent was 1.34 (0.90-1.99), and for each year of exposure to any aromatic solvent, the adjusted OR was 1.07 (1.00-1.14). Exposure to any aromatic solvent for more than three years, compared with no

exposure, was associated with a marginally significant increase in the odds of oligomenorrhea; adjusted OR = 1.53 (1.00-2.34). The adjusted OR for exposure to "all aromatic solvents" was 1.76 (1.08-2.82).

Ghosh et al., 2012. Assessing the Influence of Traffic-related Air Pollution on Risk of Term Low Birth Weight on the Basis of Land-Use-based Regression Models and Measures of Air Toxics

Ghosh et al. conducted this retrospective cohort study to examine whether measured and modeled air pollution metrics showed consistent associations with term LBW, indicating a role for traffic exhaust exposures. The authors had previously used land-use-based-regression (LUR) methods to achieve better spatial resolution in modeling traffic-related prenatal air pollution exposures, and compared LUR results with air monitoring data. The present study includes a longer study period, enabling the evaluation of both temporal and spatial exposure variations.

The authors obtained electronic birth certificate data to identify women who resided in Los Angeles County (LA) and gave birth between January 1, 1995 and December 31, 2006 (n=1,745,754). Births with recorded defects, missing or extreme gestational ages (<140 days or >320 days), extreme birth weights (<500 g or >5,000 g), or multiple gestations were excluded. Addresses were geocoded and mapped and overlaid with the geocoded locations of LA air toxics monitoring stations managed by the California Air Resources Board (CARB). Of the 1,522,267 women whose residences were geocoded, only those who resided <5 miles from a CARB air toxics station (n=415,531; 27.3%) were included in the study. Women who resided farther away from monitoring stations were excluded in an effort to balance the potential for exposure misclassification with sample size. The authors compared term LBW (<2,500 g; n=8,181) with normal weight (≥2,500g; n=370,922) term infants. Preterm births (<259 days gestation) were excluded. For the LUR analyses, a subset of 2000-2006 births (4,695 term LBW cases and 217,717 noncases) was selected.

CARB maintains three air toxics monitoring stations that were active through the entire study period, and a fourth station that provided measurements only for 2000-2006. Measurements of air toxics, including BTEX, were available for the entire study period. These measurements were used to estimate exposures for the entire pregnancy, second (days 93-185) and third (days 186-birth) trimesters, and the last month of pregnancy for air toxics and criteria pollutants (carbon monoxide, nitric oxide, nitrogen dioxide, nitrogen oxides, ozone, PM_{10}, and $PM_{2.5}$). Air toxics, including BTEX, were measured every 12 days and averaged. For the 0.9% of women who lived within 5 miles of two stations, the authors created daily averages when data were available from one or both stations, weighted by the inverse of the distance from the station.

Ghosh et al. used previously-developed LUR models to represent traffic-related air pollution. However, because these LUR models were applied only to nitric oxide, nitrogen dioxide, and nitrogen oxides, they will not be discussed in this summary.

Ghosh et al. used logistic regression to estimate ORs associated with unit or interquartile range (IQR) increases in exposure. The assumption of linearity between the log odds of term LBW and exposure was confirmed. The authors adjusted for the following confounders identified in their previous studies: maternal age, race/ethnicity, education, parity, gestational age (weeks), and gestational age squared. Mother's birthplace and a Census-based measure of socioeconomic status were also evaluated for confounding, but not included in final models.

BTEX compounds were strongly inter-correlated, with Pearson correlation coefficients ranging from 0.79 to 0.92. Pollutant estimates were also correlated across pregnancy periods (data for xylenes were not reported). The overall prevalence of term LBW was 2.2%. As shown in Table A.1, term LBW was associated with IQR increases in third trimester exposure to m,p-Xylenes and o-Xylene. Third trimester benzene and toluene exposures, and last month exposures to xylenes, benzene, and toluene (results not reported) were also associated with increased odds of term LBW. Due to small case counts and unstable estimates, results from the fourth air monitoring station were not reported. The authors state the results provide additional evidence for a contribution of traffic exhaust to risk of term LBW, and that both spatial and temporal variability are important.

Table A.1. Adjusted* odds ratios (95% CIs) for term low birth weight and interquartile range increase in estimated third trimester exposure to xylenes

CARB Stations	Number of cases/noncases	m,p-Xylenes	o-Xylene
All	7,689/346,999	1.03 (1.01-1.06)	1.03 (1.01-1.05)
North Long Beach	2,123/92,923	1.04 (0.97-1.11)	1.01 (0.97-1.06)
Burbank	1,016/53,831	1.05 (0.99-1.12)	1.05 (0.99-1.11)
Downtown Los Angeles	4,041/173,635	1.01 (0.98-1.05)	1.02 (0.98-1.06)

* Adjusted for maternal age, race/ethnicity, education, parity gestational age, and (gestational age)^2 (Ghosh et al., 2012)

Comments

Due to the high correlations among the BTEX compounds, the results for xylenes must be interpreted with caution.

The authors raise the possibility of misclassification due to residential mobility and the use of the birth certificate address. They state that while approximately 20% of LA women move during pregnancy, they typically stay in the same neighborhood.

Ghosh et al. state another source of exposure misclassification was distance from monitoring stations. When data were restricted to women within three miles of monitoring stations, Ghosh et al. found somewhat stronger associations but large

overlap in CIs, suggesting some misclassification due to residential distance from monitoring stations.

The authors note that the collection of data only every 12 days also could have caused them to miss some peaks in levels of toxics due to unusual events.

Ghosh et al. also note that excluding preterm babies introduced selection bias, because excluded preterm growth-restricted infants may have had greater exposures to air pollution.

Lehman et al., 2002. The Influence of Maternal Exposure to Volatile Organic Compounds on the Cytokine Secretion Profile of Neonatal T Cells

The purpose of this cross-sectional study was to investigate whether VOC exposure is associated with alterations of the cytokine secretion profile of cord-blood T cells. The cytokine secretion profile of T cells stimulated by environmental factors accounts for allergic responsiveness. The subjects were drawn from a cohort for an ongoing prospective study of the association between maternal exposure to VOCs and immune status at birth. The original cohort had been recruited between December 1997 and January 1999 and included only healthy full-term neonates born in Leipzig, Germany, to mothers of German descent who did not suffer autoimmune diseases or infectious disorders during pregnancy. For the present study, the authors randomly selected 85 children, analyzed cord blood T cell cytokines, and measured VOCs in their homes. Parents answered questionnaires on possible sources of VOC exposure (including painting, flooring, and smoking in the home) and family atopy history.

Within six hours of delivery, umbilical cord blood cells were stimulated using a whole blood assay with 10 ng/ml of phorbol ester and 1 µM of ionomycin in the presence of 2.5 µM of momensin for 5 hours at 37ºC and 5% CO_2. Stimulated cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Cells were simultaneously stained with fluorescence-labeled monoclonal antibodies against the T cell surface antigen CD3 and human cytokines interferon-γ-producing (IFN-γ) type 1 T cells, tumor necrosis factor-α (TNF-α), interleukin-2-producing (IL-2) T cells, and interleukin-4-producing (IL-4) type 2 T cells.

VOCs were collected by continuous passive sampling in children's dwellings for four weeks after birth. The detection limit was between 0.1 and 1 µg/m^3. Because there are no threshold values for indoor contamination with VOCs, concentrations above the 75th percentile were designated as elevated.

Lehman et al. used Mann-Whitney U-tests to analyze the data. Elevated (m+p)-xylene was significantly associated with cytokine-producing cord blood T cells in unadjusted analyses. The median IFN-γ was 3.0 for m, p-Xylene below the 75th percentile, and 4.8 for (m+p)-xylene above the 75th percentile ($p<0.05$). The median IL-4 was 0.71 for (m+p)-xylene below the 75th percentile, and 1.37 for (m+p)-xylene above the 75th percentile ($p<0.05$). No significant associations were observed for o-Xylene in

unadjusted analyses. The authors conducted logistic regression to estimate adjusted ORs for the associations between cytokine-producing cord blood T cells and all VOCs that showed associations in crude analyses, adjusting for maternal smoking during pregnancy, family atopy history, and gender. In these multivariate analyses, xylenes were not associated with cord blood cytokine-producing T cells. The authors state that the clinical relevance of altered cytokine secretion profile of cord-blood T cells in association with VOC exposure is not known.

Lindbohm et al., 1990. Spontaneous abortions among women exposed to organic solvents

Lindbohm et al. conducted a case-control study to examine the association between spontaneous abortion (SAB) and maternal occupational exposure to different types of specific solvents. The study population comprised women who were biologically monitored from 1965 through 1983 by the Finnish Institute of Occupational Health (FIOH) for at least one of the following six solvents: styrene, toluene, xylene, tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane. These biological measurements were usually done on heavy and well-documented exposures. The data from FIOH were linked to the nationwide data base on pregnancies treated in the hospital between 1973 and 1983, and the Finnish Register of Congenital Malformations from 1973 to 1982 to identify all pregnancies and SABs recorded in the Hospital Discharge Register and hospital outpatient clinic records. These registers covered 94% of all officially recorded births in 1973-1983 and an estimated 80-90% of all recognized SABs. The proportion of SABs to all pregnancies was 8.9% in the national data base. A case was defined as a woman who had a SAB; if a woman had more than one, only one was randomly selected. For each case, the authors attempted to select three controls from women who had a birth and never had a registered spontaneous abortion or child with a malformation. Controls were matched with cases on age within 2.5 years, using the nearest available matching.

Study subjects were mailed a questionnaire to obtain data on their employment, workplace, and occupational exposure during the first trimester. Subjects were asked to describe work tasks in detail, to report whether their work included specific tasks that involve solvents, whether they handled the six monitored solvents, to report other solvents handled, and the frequency of handling. Data on heavy lifting at work or home, pregnancy and work histories, health (including febrile disease), and smoking and alcohol use during the first trimester were also sought. The response rate was 85.0% among the cases and 85.7% among the controls. The pregnancy of interest was reported by 78% of SAB cases and 99% of controls. Only cases who confirmed the pregnancy and provided detail on occupational exposures during early pregnancy, and matched controls were included (73 cases, 167 controls).

Without knowledge of case status, two of the researchers classified the women's likelihood and level of exposure, based on occupation, work description, reported use of solvents, and biological exposure measurements when available. The authors were also able to confirm reported data with independent laboratory log books. The

biological monitoring data reflected short-term exposures, and only 5% of workers had been measured during the first trimester. Exposure classification was therefore based on work description and reported solvent use, with biological monitoring data supporting the information. The biomonitored levels of urinary methylhippuric acid for xylene were low in the 21 workers with measurements: median <0.1 and mean (SE) = 0.7 (0.4), with no results exceeding the Finnish Reference Value of exposed (10 mmol/l). Exposure to organic solvents in general was more common among cases (58%) than controls (42%).

Lindbohm et al. used logistic regression for individually matched data, based on the conditional likelihood function, to estimate ORs. The OR (CI) for xylene was 1.3 (0.4-4.5), adjusted for previous SAB, parity, smoking, use of alcohol, and exposure to other solvents, with five cases and seven controls exposed. The OR (CI) for SAB and solvent exposure, adjusted for previous SAB, parity, smoking, and use of alcohol, was 2.2 (1.2-4.1). Fever and heavy lifting were not included in the models because they did not change the models.

Lupo et al., 2011. Maternal Exposure to Ambient Levels of Benzene and Neural Tube Defects among Offspring: Texas, 1999-2004

Lupo et al. conducted this case-control study to assess the association between maternal exposure to environmental levels of benzene, toluene, ethylbenzene, and xylene (BTEX) and neural tube defects (NTDs; spina bifida and anencephaly) in offspring. Benzene was the primary pollutant of interest, as it has been associated with other adverse health outcomes. The authors note that Texas ranks first in the U.S. for benzene levels in ambient air and accounts for 48% of all benzene emissions in the nation.

Data on live births, stillbirths, and electively terminated fetuses with NTDs delivered from 1999 through 2004 were obtained from the Texas Birth Defects Registry, which is a population-based active surveillance system. Controls were a stratified random sample of unaffected live births delivered during the same period, selected at a ratio of four controls per case and frequency matched to cases by year because the birth prevalence of NTDs was decreasing over time. Analyses included 533 spina bifida cases, 303 anencephaly cases, and 3,695 controls.

The mother's residential address at delivery was geocoded and mapped to a census tract. Census tract-level estimates of ambient BTEX levels, reported as annual concentrations in micrograms per cubic meter ($\mu g/m^3$), were obtained from the U.S. EPA 1999 Assessment System for Population Exposure Nationwide (ASPEN), which is based on the U.S. EPA Industrial Source Complex Long-Term Model and "takes into account emissions data, rate, location, and height of pollution release; meteorological conditions; and the reactive decay, deposition, and transformation of pollutants."
The authors obtained information on the following potential confounders from vital records data: infant's sex, year of birth, maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other); maternal birth place (U.S., Mexico, other);

maternal age (< 20, 20-24, 25-29, 30-34, 35-39, ≥ 40 years); maternal education (< high school, high school, > high school); marital status (married or not married); parity (0, 1,2, ≥ 3); maternal smoking (yes or no); and season of conception (spring, summer, fall, winter). A census tract-level estimate of socioeconomic status (% below poverty level) from the 2000 Census was categorized into quartiles based on the distribution among controls.

Lupo et al. used mixed-effects logistic regression to assess associations between each pollutant and NTD phenotype while accounting for potential within-group correlation resulting from assigning exposures at the census-tract-level. As risk factors appear to be different for spina bifida and anencephaly, the authors conducted separate analyses for the two phenotypes. Because plots of the benzene levels and NTD prevalence showed a non-linear relationship, the authors used restricted cubic splines fit to the logistic regression models, with four knots corresponding to pollutant levels where the exposure-outcome relationship changed. The knots were used to determine cut points for low (reference), low-medium, medium, medium-high, and high ambient pollutant levels. Different reference exposure levels (10th, 15th, and 20th percentiles among controls) were also assessed.

Levels of BTEX were very highly correlated, $\rho^{\wedge} \geq 0.97$, $p < 0.001$; therefore, multiple pollutant models were not assessed. After adjusting for year of birth, maternal race/ethnicity, and parity, mothers who lived in census tracts with higher xylene exposure were not at significantly increased risk of having offspring with spina bifida or anencephaly. Tables A.2 and A.3 show the xylene levels, numbers of cases and controls, and corresponding ORs and CIs for spina bifida and anencephaly, respectively. ORs for the associations between xylene and NTDs were consistently but not significantly greater than one. The only significant associations were for benzene and spina bifida. No significant associations were observed for BTEX and anencephaly.

Table A.2. Xylene levels, numbers of subjects, and ORs (95% CIs) for spina bifida

Spina bifida		
Xylene levels (μg/m^3)	Cases/controls	Adjusted OR*, 95% CI
0.18– 0.36	21/190	1.00
>0.36–1.10	177/1092	1.45 (0.88, 2.36)
>1.10–1.96	164/1100	1.39 (0.85, 2.27)
>1.96–3.90	140/1133	1.18 (0.72, 1.94)
>3.90–8.84	31/180	1.64 (0.90, 3.01)

* Adjusted for year of birth, maternal race/ethnicity, and parity

Table A.3. Xylene levels, numbers of subjects, and ORs (95% CIs) for anencephaly

Anencephaly		
Xylene levels (µg/m^3)	Cases/controls	Adjusted OR*, 95% CI
0.18– 0.36	14/183	1.00
>0.36–1.12	92/1110	1.35 (0.70, 2.58)
>1.12–1.97	91/1107	1.36 (0.71, 2.60)
>1.97–3.86	92/1110	1.32 (0.69,2.52)
>3.86–8.84	14/185	1.26 (0.56, 2.85)

* Adjusted for year of birth, maternal race/ethnicity, and parity

Comments

Lupo et al. note that a potential limitation was related to exposure assessment, which was based on modeled predictions, i.e., the ASPEN model. The authors state ASPEN has been shown to be a good surrogate for measurements based on personal monitoring for benzene, but do not address its validity for xylene.

The authors state that although ASPEN data were only available for 1999, the sources of hazardous air pollutants were unlikely to change during the study period.

The authors also address the lack of information on folic acid and/or multivitamins, stating that the pregnancies in the study were conceived after mandatory folic acid fortification [of foods such as breads, cereals, flours, corn meals, pastas, rice, and other grain products] and a recent study had shown little evidence of an association between NTDs and folic acid intake since fortification.

The authors mention exposure misclassification due to using maternal residence at time of delivery, whereas the critical exposure window for NTDs is within four weeks after conception. Lupo et al. cite their own previous research showing no significant change in benzene exposure assignment when using address at delivery vs. address at conception.

Sallmén et al., 1995. Reduced fertility among women exposed to organic solvents

Sallmén et al. conducted this retrospective time-to-pregnancy (TTP) study, an extension of the study by Lindbohm et al. (1990), to investigate whether maternal occupational exposure to organic solvents is associated with reduced fertility. The study population comprised women who were biologically monitored from 1965 through 1983 by the Finnish Institute of Occupational Health (FIOH) for at least one of the following six solvents: styrene, toluene, xylene, tetracholoroethylene, tricholoroethylene, and 1,1,1-trichloroethane. For xylene, urinary methylhippuric acid concentration was measured (Lindbohm et al., 1990). These biological measurements were done because Finnish law requires regular medical examinations of workers exposed to hazardous agents. The data from FIOH were linked to the nationwide data base on pregnancies treated in the hospital between 1973 and 1983, and the Finnish Register of Congenital

Malformations. In the study by Lindbohm et al. (1990), a case was defined as a woman who had a spontaneous abortion (SAB) and a control was a woman who had had a birth and never had a registered spontaneous abortion or child with a malformation. Lindbohm et al. selected three age-matched controls per case. If a woman had more than one pregnancy, only one was randomly selected. The same pregnancies were selected for this study. Thirty additional referents were drawn from a simultaneous malformation study.

A questionnaire was mailed to 355 women to obtain data on their TTP and related factors, including whether the pregnancy was planned, frequency of intercourse, and for those not using contraception at the time of pregnancy, how many menstrual cycles the woman had not used contraception before getting pregnant (in classes of menstrual cycles as follows: 1 cycle, 2 cycles, 3-4 cycles, 5-6 cycles, 7-12 cycles, 13-24 cycles, 25+ cycles). They were also asked whether and for how long before the study pregnancy they had stopped using an intrauterine device, oral contraceptives, or spermicides. Subjects were also asked questions about breast feeding, stress, and lifestyle factors such as smoking, alcohol and caffeine consumption, partner's smoking and history of illness or other condition that might have affected his fertility. Data on heavy lifting at work or home, pregnancy and work histories, health, and smoking and alcohol use during the first trimester were also sought. After three mailings, 235 (66%) women participated; of these, 197 subjects remained after exclusions.

Subjects completed mailed questionnaires which asked them to describe work tasks in detail, to report whether their work included specific tasks that involve solvents, whether they handled the six monitored solvents, to report other solvents handled, and the frequency of handling. Two of the researchers, blinded to pregnancy outcome, classified the women's likelihood and level of exposure, based on occupation, work description, reported use of solvents, and biological exposure measurements when available. The same exposure assessment was used for the TTP and SAB studies if the woman had the same job during TTP as during the first trimester of the study pregnancy. If the work tasks had changed, two researchers classified each work task by likelihood and level of exposure, without knowledge of TTP. The biological monitoring data reflected short-term exposures, and only 5% of workers had been measured during the first trimester. Exposure classification was therefore based on work description and reported solvent use, with biological monitoring data supporting the information. For xylene, the measured levels were low, with no levels exceeding the Finnish Threshold Limit Value.

The authors used discrete proportional hazards regression to analyze the TTP data, with the ~10-day periovulatory period within menstrual cycles representing the disjointed time intervals in the background of the model. The outcome is an estimate of the ratio of average incidence densities of clinically recognized pregnancies for exposed vs. unexposed women in each menstrual cycle class. The incidence density ratios (IDRs) for women who were employed at the beginning of TTP and had low and high exposure to organic solvents, vs. none, were 0.74 (95% CI 0.49-1.11) and 0.44 (95% CI 0.28-0.70), respectively, adjusted for age, previous induced abortion or extrauterine

pregnancy, recent use of IUD, alcohol use, older age at menarche, unplanned pregnancy, and frequency of intercourse. These IDRs suggest that greater exposure to organic solvents resulted in longer TTP and reduced fecundability. The IDRs for xylene are shown in Table A.4.

Table A.4. Adjusted Incidence Density Ratios* of clinically recognized pregnancies and exposure to xylene

Exposure level	N	Adjusted Incidence Density Ratio (95% CI)
Low	31	1.41 (0.91-2.20)
High	10	0.93 (0.47-1.84)

* The models include exposure to other solvents, recent contraceptive use, and age at menarche. The referent category was not exposed. (Sallmén et al., 1995)

Comments

Sallmén et al. evaluated the potential effects of response bias and infertile worker effect and found that the associations persisted for high exposure to solvents but were weaker for low exposure.

The participation rate in this study was quite low, with varying levels of participation according to age, SAB case status, exposure, and parity.

Most of the women exposed to aromatic hydrocarbons were simultaneously exposed to other solvents.

TTP data were collected 8-18 years after the pregnancies with a self-administered questionnaire.

Sallmén et al., 1998. Time to pregnancy among the wives of men exposed to organic solvents

Sallmén et al. (1998) conducted this retrospective study to investigate whether paternal exposure to organic solvents (styrene, toluene, xylene, tetracholoroethylene, tricholoroethylene, and 1,1,1-trichloroethane) is associated with delayed conception. This study is an extension of a case-control study of the effects of paternal exposure to organic solvents on spontaneous abortion (SAB) and congenital malformations, by Taskinen et al. (1989).

The source population was male workers who had been monitored for organic solvent exposure by the Finnish Institute of Occupational Health from 1965 through 1983. The authors obtained information about the men's marriages and wives through the Finnish Population Register Center, and information about pregnancy outcomes through pregnancy outcome registers. Men in their first marriages at the time of the first inquiry (1985) and whose wives were 18-40 years of age at the end of the first trimester of pregnancy, and pregnancies that had begun during or up to nine months before the

marriages were included. The study subjects were cases and controls from the initial study, *i.e.*, couples who had had a SAB or a child with a malformation, and couples who had not had a SAB or a child with a malformation, matched by age at a ratio of 3:1 for SAB and 5:1 for MALFORMATION. Only one pregnancy per woman was included. Four hundred thirty-eight couples from the initial study were contacted for the time to pregnancy (TTP) study, 316 (72%) couples responded, and 282 couples met all inclusion criteria and were included in final analyses.

Questionnaires were mailed to the wives of the case and control families six years after the first study (8-18 years after the pregnancies). Women who had not been using contraception were asked questions pertaining to TTP, whether the pregnancy was planned, contraception history, frequency of intercourse, breastfeeding, pregnancy history, heavy lifting, febrile or inflammatory diseases, endometriosis, abdominal surgery, treatment for infertility, age at menarche, menstrual cycles, and lifestyle factors such as smoking and use of alcohol or caffeinated beverages during the pregnancy. Women were also asked for information on occupational and lifestyle exposures, and questions related to the husband's smoking and conditions that may have affected his ability to become a father.

Classification of the husband's exposure to solvents was generally based on the self-reported occupation, job description, reported solvent or other chemical usage during the year the pregnancy started, and biomonitoring data (available for 69% of men), that had been collected for the previous study, without knowledge of the time to pregnancy. The exposure assessment from the previous study was adopted if the husband had the same job at the beginning of the TTP as during the 80 days (estimated period of spermatogenesis) before the beginning of the study pregnancy. A new assessment was made for men whose work tasks had changed during the TTP. Chemical composition of commercial products was checked from the safety sheets or the Register of Safety Information on Chemical Products. Likelihood of exposure was categorized as unexposed (no reported exposure and not monitored in the same job), potentially exposed (job description implied exposure but no clear solvent exposure was reported and no exposure measurement was conducted), or likely exposed (the job implied solvent exposure and there were exposure measurements from the same job, or solvent exposure was reported).

The exposure levels were categorized as follows:

- high or frequent (for daily handling of solvents, or if biological exposure measurements indicated exposure above the reference values for the general population)
- intermediate or low (exposure 1-4 days/week and the biological measurements indicated low exposure)
- none

For xylene exposure, there were 70 men in the low/intermediate category and 51 in the high category. The appendix of the study reports biological measurements for 11 men in the high xylene exposure group and two men in the low/intermediate xylene exposure

group, with a footnote stating that measurements with urine specific gravity < 1.008 were excluded. The reported median urinary methylhippuric acid levels were 0.36 mmol/l in the high exposure group, with 72.7% of subject samples exceeding the Finnish reference value of 10.0 mmol/l, and 0 mmol/l in the low/intermediate group.

Data were analyzed using discrete proportional hazards regression, with the outcome interpretable as an incidence density ratio – in this case, the fecundability density ratio (FDR), or [fecundability of the exposed]/[fecundability of the unexposed]. The FDR (CI) was 0.74 (0.51-1.06) for low/intermediate exposure to solvents; and 0.80 (0.57-1.11) for high/frequent exposure, adjusted for maternal age, age ≥ 15 at menarche, menstrual cycle variables, frequency of intercourse, maternal and paternal smoking, maternal exposure to organic solvents, year of pregnancy, and a variable to control for missing information. For xylene, the FDRs were similar: 0.75 (0.52-1.09) for low/intermediate exposure and 0.91 (0.61-1.36) for high/frequent exposure, adjusted for short menstrual cycle, long or irregular menstrual cycle, older age at menarche, frequency of intercourse, maternal age, maternal exposure to organic solvents, and a variable for missing information. These FDRs suggest that exposure to solvents and xylene might be associated with reduced incidence of pregnancy, though the findings were not statistically significant.

Comments
The authors note that simultaneous exposure to multiple solvents was common and risks cannot be attributed to specific solvents.

This study used a study population from a case-control study of SAB, with 23% of pregnancies ending in SAB. The authors note that such pregnancies tend to have longer TTP than those ending in births. Sallmén et al. further state that the data on TTP might not be as accurate for SABs as for births, thus potentially biasing the FDR for exposure to solvents.

Because this retrospective study included only couples that achieved pregnancy, infertile couples were excluded. The authors note that older couples who had pregnancies before the study period might be underrepresented because for some of them, their fertile period only partly overlaps with the study period. If solvent exposure is associated with sterility or long TTP, this study could be biased toward the null.

Swan et al., 1995. Historical Cohort Study of Spontaneous Abortion Among Fabrication Workers in the Semiconductor Health Study: Agent-Level Analyses

The Semiconductor Health Study was a retrospective cohort study designed to examine the relationship between occupational exposures in semiconductor manufacturing and risk of spontaneous abortion (SAB). There were three levels of analysis: 1) fabrication room (fab) vs. nonfab work; 2) analyses of two super-groups based on work tasks; 3) agent-specific analyses, the subject of the study summarized here.

Women 18-44 years of age were identified through company records and screened by telephone to identify those who became pregnant in 1986-1989 while working at one of 14 semiconductor companies. The final sample included 506 current and 385 former employees. Work history and other exposures during the first trimester of pregnancy were ascertained through interviews. SAB was defined as a pregnancy terminated by 20 weeks' gestation, except ectopic and molar pregnancies, and elective terminations. SABs were ascertained in interviews and confirmed by medical record review or physician interview when possible. The outcome measure was the SAB rate, defined as the number of SABs divided by the sum of live births, SABs, and stillbirths.

Each pregnancy was classified as fab or nonfab and was classified into one or more work groups, depending on the woman's job activities at conception. The authors also classified first trimester exposure to specific agents which were selected by industrial hygienists based on evidence for reproductive toxicity and prevalence of exposure. These agents included 12 groups of chemicals, 2 physical agents, radiofrequency radiation, and extremely low-frequency magnetic fields. Chemical agents were also examined in functionally similar subgroups: photoresist and developer solvents (PDS; including xylene), fluoride compounds, cleaning solvents, and dopants [substances such as arsenic, phosphorus, boron, and antimony, introduced into semiconductors to modulate electrical properties]. Exposure scores were calculated for each agent based on each woman's self-reported work activities and hours, and in the case of fab workers, the industrial hygienists' evaluations of the fab where the woman worked. Exposure scores were grouped into four levels ranging from no exposure to highest (0-3). The cohort included 683 unexposed women and 189 who were exposed to xylene. Workplace stress, age, smoking, ethnicity, pregnancy history, and socioeconomic status (income and education) were also evaluated.

The authors conducted logistic regression analyses and converted ORs to RRs. The referent groups were all workers who were not exposed to a given agent or group of agents, regardless of whether they were fab workers. Pregnancy year was included in analyses because of the lower SAB rate during the early study years and the changing workforce composition. When considered singly, xylene was among the seven agents identified as strongly associated with SAB, with higher rates and RRs in the middle and high exposure groups (Table A.5).

Table A.5. SAB rates and unadjusted RRs (95% CIs)* by xylene exposure

Exposure level	SAB rate (n)	RR (95% CI)
0	0.12 (79/683)	-
1	0.12 (14/117)	1.04 (0.61-1.77)
2	0.23 (9/40)	1.95 (1.03-3.67)
3	0.31 (10/32)	2.70 (1.50-4.88)

* Chi-square test for trend, p=0.0008 (Swan et al., 1995)

Because most women who worked in fabs were exposed to multiple, highly correlated agents, the authors were unable to obtain convergence for a logistic model with all

agents simultaneously, so they combined workers with similar exposures into functional subgroups. Women who were exposed to xylene in masking activities (including photolithography and etching), vs. non-fab workers, were at increased risk of SAB, while women working with dopants and thin film were not at significantly increased risk (Table A.6).

Table A.6. Adjusted* RRs (95% CIs) for xylene and SAB, by workgroup

Xylene exposure level**	All women	Masking vs. non-fab (excluding dopants and thin film)	Dopants and thin film vs. non-fab (excluding masking)
1-3	1.35 (0.88-2.03)	1.51 (0.92-2.36)	1.47 (0.40-4.16)
2-3†	2.31 (1.39-3.58)	2.72 (1.51-4.37)	2.01 (0.42-5.86)

* Adjusted for smoking, age, education, income, ethnicity, pregnancy history, pregnancy start year, stress question
** Exposure level 0 is the referent
† Women at exposure level 1 are excluded from analysis (Swan et al., 1995)

Exposures to xylene and n-butyl acetate (nBA) could not be separated because all women exposed to nBA were also exposed to xylene, and all but 12 women exposed to xylene were exposed to nBA. Moreover, most women exposed to xylene and nBA were also exposed to ethylene-based glycol ethers (EGE), which were also associated with SAB. Thus, the associations between xylene and SAB may reflect EGE exposure. Swan et al. also estimated the RR for exposure to any PDS and found a strong association when at least one exposure (xylene, EGE, PGE, or nBA) was at level 3 (RR = 2.70 (1.40-4.55)), and little association when the highest exposure was at level 1 (RR=0.89 (0.49-1.55)). The authors state that the data suggest a threshold for PDS with increased risk of SAB only at exposure level 2 or 3.

Taskinen et al., 1989. Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents

Taskinen et al. conducted a nested case-control study to investigate the effects of paternal exposure to organic solvents styrene, toluene, xylene, tetracholoroethylene, tricholoroethylene, and 1,1,1-trichloroethane on pregnancy outcomes. The source population was workers who had been monitored for organic solvent exposure by the Finnish Institute of Occupational Health from 1965 through 1983.

The authors obtained information about the men's marriages and wives, including personal identification numbers, through the Finnish Population Register Center, and information about pregnancy outcomes through pregnancy outcome registers. The study included only men in their first marriages in 1985, their wives if they were 18-40 years of age at the end of the first trimester, and pregnancies that had begun during or up to nine months before the marriage. Data on medically diagnosed pregnancies were obtained from the Hospital Discharge Register. Data on SABs treated in hospital

polyclinics during 1973-1983 [presumably the “outpatient clinics” referenced in Taskinen et al., 1994] were collected separately from the hospitals. A case was defined as a wife who had a SAB or a child with a congenital malformation. If a wife had more than one SAB, one was randomly selected for the study.

Data on malformations during 1973-1982 were obtained from the Finnish Register of Congenital Malformations. Reporting congenital malformations identified in the first year of life is mandatory. However, the authors note that studies comparing register data with hospital data have shown a reporting and detection failure rate of approximately 30%; minor malformations are particularly underreported.

Three controls for each SAB and five controls for each malformation were selected from the wives who had given birth and had not had a SAB or a child with a malformation during 1973-1983. Controls were matched to each case by age at time of conception as closely as possible and within 30 months, and only one pregnancy per woman was included. The final SAB study sample included 120 cases and 251 controls, with response rates of 79.1% and 73.3%, respectively. The final malformation study sample included 25 cases and 96 controls, with response rates of 75.8% and 75.0%, respectively.

Questionnaires were mailed in January 1986 to spouses of both the case and control families to obtain data on occupational exposures, including the men’s employment, occupation, and workplace during the year of conception. Men were asked to describe their work tasks and any changes in them in detail, whether they had handled any of the monitored solvents or other solvents, and the frequency of handling (daily, 1-4 days/week, < once a week). Almost 300 trade names of solvents were reported. Chemical composition of commercial products was checked from the safety sheets or the Register of Safety Information on Chemical Products. Frequency of exposure to gases, vapors, dusts, fumes, and other chemicals, or radiation in the work was also requested, as was information on earlier employment, chronic diseases, smoking, and alcohol consumption. If sufficient information was not obtained in the first questionnaire, a new questionnaire was mailed. Wives were also asked for information on occupational and lifestyle exposures, as well as pregnancy history, heavy lifting and febrile diseases. Only couples who reported the pregnancy reported in the registers were included in the analyses.

The husband’s exposure during estimated spermatogenesis (80 days) for the pregnancy was assessed based on the occupation, job description, reported solvent or other chemical usage, and biomonitoring data, without the case status being known. Likelihood of exposure was categorized as follows:

- Unexposed – no reported exposure and not monitored in the same job
- Potentially exposed – solvent usage was possible, based on the job description, but no clear solvent exposure had been reported and no exposure measurement had been conducted

- Likely exposed – the job implied solvent exposure and there were exposure measurements from the same job or solvent exposure was reported.

Biomonitoring measurements included methylhippuric acid in the urine for xylene. Although many measurements of the six solvents or metabolites were taken, only a few measurements were conducted during the time of spermatogenesis (17 measurements) or the preceding year (44 measurements), and Taskinen et al. state that biological measurements only indicate exposure on the same or previous day. Thus, exposure classification was based mainly on job descriptions, "and monitoring results supported the information." Exposures were defined as follows:

- "High/frequent" if the worker handled solvents daily or if the biological measurements indicated clear occupational exposure, *i.e.*, above the reference values for the general population;
- "Intermediate" when the solvent was used 1-4 days a week and the biological measurements indicated intermediate/low exposure;
- "Low/rare" if the solvent was handled more rarely.

Foremen who did not take part in the manufacturing process but were present in the work area were classified in the category below the one that would have been assigned based on the frequency of exposure. If a worker was likely exposed to solvents in general, but no specific exposure was identified, he was categorized as "exposed to solvents in general" for the final analysis, but for the purposes of the specific solvent variable, he was categorized as unexposed. Workers who reported exposure to unspecified "thinners" were classified as likely exposed to miscellaneous solvents and potentially exposed to xylene and toluene.

Women's exposure was assessed similarly, but based on the questionnaire data only. Lifting heavy burdens during the first trimester was assessed and categorized according to the weight and frequency of lifts.

The authors conducted conditional logistic regression for individually matched data.

SAB

For SAB, response rates were 79.1% for cases and 73.3% for controls, with variation among subpopulations monitored for specific solvents. Among respondents, 120 (88.2%) cases and 251 (67.8%) controls reported the pregnancy of interest and were included in analyses. Exposure to xylene was considered likely for 37 cases and 61 referents. The OR (CI) for likely paternal exposure to xylene and SAB was 1.8 (1.1-3.2) when no potential confounders were included in the model. However, no category of xylene exposure was statistically significantly associated with SAB, and each of the ORs associated with the three levels was less than 1.8. Table A.7 shows ORs for SAB and likely paternal exposure to xylene.

Table A.7. Odds Ratios and CIs for likely paternal exposure to xylene and spontaneous abortion

Exposure category	Cases (n)	Controls (n)	OR	CI
Low/rare*	7	13	1.2	0.4-3.3
Intermediate*	11	19	1.7	0.7-4.2
High/frequent*	19	29	1.6	0.8-3.2
Any (unadjusted)	37	61	**1.8**	**1.1-3.2**

* Adjusted for potential paternal exposure to the solvent, likely paternal exposure to other organic solvents and dusts, maternal exposure to solvents, maternal heavy lifting, and history of previous SAB

Likely paternal exposure to organic solvents in general was associated with SAB: OR = 2.7 (1.3-5.6). After adjustment for paternal exposure to dusts, maternal exposure to organic solvents, maternal heavy lifting, and history of SAB, the OR for paternal exposure to organic solvents was reduced somewhat: OR = 2.3 (1.1-5.0). The authors state xylene does not significantly increase the OR for SAB.

Congenital malformations
For malformations, response rates were 75.8% for cases and 75.0% for controls. All respondents confirmed the study pregnancy. Analyses included all 25 cases that were identified, as well as 96 referents. The authors report paternal exposure to organic solvents for 72% of cases and 73% of referents; no maternal exposure to organic solvents was reported for cases. The unadjusted OR for xylene and congenital malformations (excluding luxations of the hip) was 1.6 (0.4-5.7). No adjusted analyses were reported for malformations, and no significant associations were reported for malformations, for any of the solvents examined.

Comments
Taskinen et al. state that although the high prevalence of solvent exposure increased the statistical power to detect excess risk associated with exposure, the small number of unexposed cases was disadvantageous and hindered many of the relevant comparisons. To illustrate, only 14 of 120 SAB cases were classified as unexposed to solvents.

Most exposed workers were exposed to combinations of solvents. Of the 48 men highly/frequently exposed to xylene, 11 (23%) were thought to be exposed to xylene alone and 37 were exposed additionally to toluene and/or “miscellaneous” solvents.

The authors note that workers were additionally exposed to solvents other than those mentioned, indicating a complex multi-exposure situation for workplaces.

The authors also note that given the multiple comparisons made, some findings could be statistically significant due to chance.

The recall period for the earliest pregnancies was 14 years.

Taskinen et al., 1994. Laboratory Work and Pregnancy Outcome

Taskinen et al. conducted this case-control study to further elucidate the possible risks of SAB, congenital malformations, and reduced birth weight associated with laboratory work and to identify individual chemical or physical agents potentially harmful to pregnancy. Study subjects were women 20 to 34 years of age at the beginning of the study pregnancy and were sampled from 9,186 women in three overlapping sources of occupational data:

- 5,908 employees who held jobs with titles that included "laboratory" or occupations termed "assistant" in 1970 and 1975 to 1986 were drawn from the payroll of state-employed laboratory personnel in Finland (including university and research center laboratories);
- 2,426 members and previous members of the Finnish Union of Laboratory Assistants in 1987;
- 2,734 laboratory employees registered as Employees Occupationally Exposed to Carcinogens in 1979-1986.

Data on pregnancy outcomes were obtained from the Hospital Discharge Register and the database of SABs that were treated at outpatient clinics of the hospitals. SAB cases were defined as women treated for SAB during the study period; women who had more than one SAB during the study period were excluded. A malformation case was defined as a woman with a child registered in the Finnish Register of Congenital Malformations. Controls for both outcomes were selected from the women who had given birth to a child not registered in the Register of Congenital Malformations, and did not have any registered SABs. Controls were selected at a ratio of 2:1 for SAB and 4:1 for malformations, and matched to cases on age at conception and year of pregnancy. One pregnancy per woman was included. During 1973-1986, the subjects had 7,316 pregnancies, with 5,663 (77%) births, 687 (9%) SABs, and 966 (13%) induced abortions.

Subjects provided information on occupational exposures, health status, medication, contraception, smoking, alcohol consumption during the first trimester, pregnancy history, and birth weight and child's sex, by mailed questionnaire. The authors assessed exposure to individual chemicals according to the frequency of use, as reported in the questionnaires. Exposure was considered rare if a chemical was handled 1-2 days/week, and frequent if handled at least 3 days/week. For solvent exposure, two occupational hygienists who were unaware of the subjects' pregnancy outcomes and were knowledgeable about laboratory working conditions assessed the overall exposure to organic solvents based on the description of the work tasks and the use of solvents, including estimates of quantity and duration of use, and the use of a fume hood. The authors calculated an exposure index (EI), which was a multiple of quantity of solvent, time of use, frequency of use, and use of a fume hood, but did not report results that clearly included the EI.

For the SAB study, questionnaires were mailed to 1,000 women; 80.0% of the 335 cases and 83.6% of the 665 presumed controls responded. After excluding those who

did not confirm the study pregnancy, provided insufficient information, had no matched case or control, or were ineligible for other reasons, there were 206 cases of SAB and 329 controls.

For the malformation study, 248 questionnaires were mailed; 82.0% of 50 cases and 86.8% of 198 potential controls responded. After excluding those who provided insufficient information, had no matched case, or were ineligible for other reasons, there were 36 cases of malformation and 105 controls.

Conditional logistic regression for individually matched data was used to estimate ORs for SAB and malformations. Birth weight was analyzed using linear regression. Table A.8 shows ORs for xylene and SAB. SAB was associated with frequent (≥3 days/week) exposure to xylene in the first trimester: OR 3.1, 95% CI 1.3-7.5. Simultaneous exposure to multiple solvents was common; only two cases and referents were exposed to xylene alone. Similarly, the risk of SAB associated with exposure to aromatic hydrocarbons was also increased (OR 2.7, CI 1.3-5.6). Taskinen et al. also report an increase in risk associated with high values of the "solvent score" (OR 2.3, CI 1.1-4.3); whether the solvent score is the EI is not clear.

Table A.8. SAB and exposure to xylene in the first trimester: adjusted odds ratios (95% CIs) and numbers of subjects

Exposure	Adjusted* OR (95% CI)	No. of cases/controls
1-2 days/wk	1.3 (0.7-2.5)	19/27
3-5 days/wk	**3.1 (1.3-7.5)**	**16/12**

* Adjusted for employment, smoking, alcohol consumption, parity, previous miscarriages, failed birth control, and febrile disease during pregnancy

Taskinen et al. found that employed women were at significantly reduced risk of malformations. Women exposed to aromatic solvents were at non-significantly reduced risk of malformations. No associations between occupational exposures to xylene and malformations were reported.

The authors report a significant association between increased birth weight and "exposure at the most 2 days a week to xylene," but provide no data or measures of association.

Comments
The authors note that sole exposure to xylene is uncommon, and because of the simultaneous exposures to multiple solvents and chemicals, results for individual chemicals should be interpreted cautiously.

Excluding women with multiple SABs might have introduced a bias toward the null if occupational solvent exposures increase risk of SAB.

Exposure data were collected only for the first trimester, which may not be adequate to assess risks associated with birth weight.

Few details concerning the malformation analyses, and no data for the birth weight analyses, were provided.

Windham et al., 1991. Exposure to Organic Solvents and Adverse Pregnancy Outcome

The purpose of this case control study was to examine the risks of spontaneous abortion (SAB) associated with solvent exposure defined on different levels, including occupational and specific solvent exposure, elicited by both chemical solvent names and generic solvent-containing products. Fetal growth restriction was a secondary outcome of interest.

Cases were defined as women at least 18 years of age who had a SAB by 20 weeks gestation, for which a pathology specimen was submitted to one of 11 hospital laboratories in Santa Clara County, California from June 1986 to February 1987. For each case, two controls were randomly selected from county residents who had a live birth, with frequency matching by last menstrual period (± 1 week) and hospital. Of 852 cases and 1,618 controls contacted, 9.7% of eligible cases and 8.5% of eligible controls refused to participate. The final sample included 626 cases and 1,300 controls. Subjects were interviewed using computer–assisted telephone interview. Cases were asked about the entire pregnancy, whereas controls were asked only about the first 20 weeks.

Detailed information for up to two jobs during pregnancy (or the first 20 weeks for controls) was obtained and industry and occupation codes were assigned. Respondents were asked whether they regularly (at least once per week) used or worked around any of 10 solvents or commonly used solvent-containing products, as well as an open-ended category for other solvents and degreasers. Women working in electronics manufacturing were also asked about the use of other solvent-containing products. For each product used, the subject was asked the number of hours per week and weeks of exposure, whether she had skin contact, smelled odors, or experienced symptoms (headaches, dizziness, forgetfulness). Exposed women were asked about workplace ventilation, use of masks and respirators, and whether they had mixed or cleaned up spills of solvents. Only use during the first trimester was examined, based on the first week of use. Trade names were solicited and examined to verify solvent content. The authors decided a priori to exclude acetone and isopropyl alcohol due to their ubiquitous use in consumer products. All women were also asked about the non-occupational use of eight solvent-containing products and any “other” solvent. Analyses of occupational solvent use were limited to the 70% of respondents who were employed. Women were defined as non-exposed if they did not use any of the named solvents and if they did not work in the microelectronics industry. To increase numbers, solvents were collapsed into 5 classes, including aromatic.

Windham et al. used stratified analysis to assess effect modification and confounding. Confounders were controlled in logistic regression models for use of any solvents and for solvent classes. Nine cases and 12 controls reported exposure to xylene. The crude OR (CI) for xylene and SAB was 1.6 (0.66-3.8). To examine the effect of "intensity" of exposure, Windham et al. analyzed exposures which involved skin contact, odor, or symptoms, vs. those that did not. The OR for xylene exposures that involved skin contact, odor, or symptoms was 1.6 (p=0.23) and the OR for lower intensity exposure was 1.5 (p=0.52). No adjusted OR for xylene was reported. The OR for aromatic solvent exposure was 1.2 (0.57-2.4), adjusted for maternal age, race, prior fetal loss, education, smoking, average hours worked, and interviewer-rated data quality. The adjusted OR for aromatic solvent exposure for 1-10 hours/week was 2.7 (0.96-7.4), and for >10 hours/week the adjusted OR was 0.56 (0.15-2.1).

Windham et al. also examined the association between solvent exposure and fetal growth in liveborn controls. For occupational aromatic solvent exposure and intrauterine growth restriction, defined as less than the tenth percentile of weight for gestational week, the crude OR was 1.9 (0.53-6.6), with three cases and 20 controls exposed. The crude OR for occupational aromatic solvent exposure and low birth weight (<2,500 g) was 0.60 (0.08-4.6), with one case and 22 controls exposed. No results for xylene were reported.

Comments
Very few subjects were exposed to xylene.

Control of confounding was limited by the small numbers of subjects when specific exposures were examined.

Xiao et al., 1999. Effect of benzene, toluene, xylene on the semen quality of exposed workers

Xiao et al., 2001. Effect of Benzene, Toluene, Xylene on the Semen Quality and the Function of Accessory Gonad of Exposed Workers

Xiao et al. (1999 and 2001) conducted this cross-sectional study in June 1994-July 1996 in a city in Zhejiang Province in order to examine the effects of BTX on semen quality of workers. The authors selected married workers who had at least one child and at least one year of experience working in shoemaking, spray painting, or paint manufacturing, and were exposed to high airborne concentrations of BTX. Of these 56 "exposed" workers, 24 agreed to donate blood and semen samples. The authors selected 40 "age- occupational-matched non-exposed controls with similar physical activity" from managers; 37 agreed to provide samples. The source population and subject selection and recruitment procedures are not described further.

Subjects were interviewed about reproductive history, tobacco and alcohol use, and detailed occupational and medical histories. Xiao et al. state that there were no significant differences in the characteristics of the two groups.

The mean concentrations in work air were: benzene 103.34 (0-7070.3) mg/m^3, toluene: 42.73 (0-435.8) mg/m^3, and xylene 8.21 (0-133.1) mg/m^3 (the numbers in parentheses are not explained).

Participants were asked to abstain from ejaculation for 48 hours before sample collection, which was on site. Semen samples were delivered to the laboratory immediately and incubated at 37º C until liquefaction completed. Whole semen analyses included liquefaction time, semen pH, sperm concentration, total sperm count, percentage vitality (2001), and sperm activity, and was completed within one hour of liquefaction. Additional analyses of acrosin activity (believed to be important for fertilization), seminal fructose (provides energy for sperm activity), seminal γ-glutamyltransferase (γ-GT) activity (reflecting prostate function), and relative activity of lactate dehydrogenase C4 (LDH-C4; important for sperm motility) were also conducted. Blood and semen samples were analyzed for BTX using headspace chromatography. BTX was detected in samples from some of the exposed workers, but not in samples from the control workers. Table A.9 shows biomonitoring results for workers who had detectable BTX in blood and/or semen samples.

Table A.9. Results of biomonitoring in exposed workers who had positive findings for BTX (geometric mean, µmol/L)

Sample	N	Benzene		Toluene		Xylene	
		# positive	mean (range)*	# positive	mean (range)*	# positive	mean (range)*
Blood	24	13	4.40 (0.40-51.32)	11	1.42 (0.30-17.17)	11	1.32 (0.38-4.50)
Semen	17	12	1.85 (0.17-8.54)	6	0.22 (0.11-0.40)	10	5.67 (1.12-33.84)

*For positive samples only (from Xiao et al., 2001 and 1999)

The authors conducted chi-squared and student's t tests and multiple regression to evaluate the relationships between BTX exposures and semen parameters. The percentages of semen samples with liquefaction time exceeding 30 minutes, volume less than 2 ml, and 75% or less vitality were higher in exposed workers (1999). Multiple regression analysis showed that blood xylene was significantly associated with decreased γ-GT (p=0.0002) (2001); however, the interpretation of the statistics reported is unclear: Beta=-30.2810, B=7.5835, SE B [not reported], Constant=-0.4086, Sig T=0.0002. Xiao et al. described the statistics as follows "Beta: standard regression coefficient; B: biased regression coefficient; SE B: standard error of biased regression coefficient; Sig T: level of significance" (1999). Whether the association for xylene was independent of potential effects of other solvents is also unclear.

γ-GT was also associated with semen benzene (p=0.0012) (2001). Sperm activity and vitality, acrosin activity, and LDH-C4 % were significantly lower according to working duration/history (1999, 2001). In regression analyses, control workers' exposure was assumed to be zero. The authors did not further describe the regression models or how to interpret them.

Comments

The authors state that they included only exposed workers who had at least one child in order to avoid selection bias. However, if BTX reduces fertility or otherwise affects fertility-related parameters, this selection could bias results by disproportionately excluding those who were exposed but adversely affected.

There is potential for bias resulting from the differences between the two samples. First, the unexposed workers were all managers. Managers could tend to have very different characteristics compared to exposed workers. For example, although the authors state that there were no significant differences in the characteristics of the two groups, the exposed workers appeared to both drink and smoke somewhat more heavily, and had shorter marriage and work histories than unexposed workers, despite being slightly older. The differences were not statistically significant, possibly due to the small sample size.

Another cause for concern about comparability between the two groups is the low participation rate among the exposed vs. unexposed workers: 24/56 (42.9%) for the exposed, vs. 37/40 (92.5%) for the unexposed. Xiao et al. state that the reason for a higher participation rate in the unexposed men is that they were volunteers, and that the "attitude of unexposed men is different from the exposed" (1999). The low participation rate among the exposed is itself also a concern.

Among the 24 subjects who were classified as exposed, less than half of blood and semen samples had detectable BTX.

The finding for xylene and γ-GT was based on no more than 11 subjects who had xylene in their blood.

The authors point out that BTX are used mostly in mixed form.

Appendix B. Summaries of Animal Studies on Xylene

In this appendix, the animal toxicological studies discussed in the main document are summarized and arranged in alphabetical order by the first author's last name and year of publication.

Biodynamics, 1983 122

Hass and Jakobsen, 1993. 128

Hass et al., 1995 132

Hass et al., 1997. 1355

Hudak and Ungvary, 1978 139

Kükner et al., 1997 141

Litton Bionetics, 1978 142

Marks et al., 1982 144

Mirkova et al., 1979 148

Mirkova et al., 1983 150

Nylen et al., 1989. 1522

Rosen et al., 1986 154

Saillenfait et al., 2003 155

Ungvary et al., 1980 158

Ungvary et al., 1981 161

Ungvary and Tatrai, 1985 162

Washington et al., 1983 166

Yamada, 1993 167

Biodynamics, 1983. Prenatal and fetal reproduction inhalation toxicity study in rats with mixed xylenes

In a large one-generation reproductive toxicity study, male and female CD rats were exposed to mixed xylenes by chamber inhalation during a 131-day pre-mating period and 20 days of mating. Pregnant females continued on their treatment regimen throughout gestation and lactation, excepting for the days between GD 20 and PND 5. Experimental treatment conditions were as follows:

- Exposure levels were 0, 60, 250, and 500 ppm
- Technical grade mixed xylenes composed of: 2.4% toluene, 12.8% ethyl benzene, 20.3% p-xylene, 44.2% m-xylene, 20.4% o-xylene
- Animals were placed in the chambers daily, for six hrs/day. Lactating females were separated from their litters during the treatment periods.
- The animals were divided into six groups
    - A chamber-exposed, sham-air control group of 30 males and 60 females (group I)
    - The 60 and 250 ppm dose groups each consisted of 10 males and 20 females (groups II and III)
    - There were three high-dose groups (500 ppm)
        - 20 males and 40 females, both sexes treated (group IV)
        - 10 males and 20 females, only males treated (groupV)
        - 10 males and 20 females, only females treated (group VI)

The evaluation scheme was as follows:

- Reproductive performance was evaluated through mating performance and pregnancy data
- 20 females from group I (sham controls) and 12 females from group IV (500 ppm) were sacrificed on GD 21 for fetal evaluations (teratology component)
    - external malformations
    - internal, soft tissue malformations
    - skeletal malformations
- Remaining pregnant females from all groups were allowed to deliver normally
    - On PND 4 pups were pooled and randomly reassigned to lactating dams with litters born on the same day in the same treatment group; where possible, litters were normalized to four ♂ and four ♀ each.
    - If only one litter was available on a given day, it was culled to a maximum of eight pups.
    - Pups were evaluated for growth and development during the lactation period
- All adult F0 animals were evaluated postmortem
    - Half of the F0 males from each group were sacrificed following the mating period; reproductive tissues were evaluated microscopically
    - Remaining males were sacrificed at three weeks post-mating, during which time they were not treated

- F0 females were sacrificed at GD 21 or PND 21 (weaning, or the end of lactation), according to the protocol
- F0 females that had not successfully mated or delivered a litter following the initial mating period were entered into a second co-habitation period. These females were sacrificed at parturition, or post-mating if they again failed to become pregnant.

- One pup of each sex from each litter was randomly selected for sacrifice and evaluation on PND 21
- Remaining offspring were weighed and sacrificed on PND 49; one pup per sex per litter was subjected to full necropsy

Results for adult (F0) toxicity were as follows:

- No mortality was seen among the treated groups; among controls, one male and two females died, while a third female was sacrificed moribund
- No adverse effects on body weight, gross postmortem evaluation, weight or histology of male reproductive organs, and no clinical symptoms of toxicity
- Group IV females showed an increase in mean kidney weight, and increased mean corrected body weight at GD 21; mean kidney to body weight ratio showed a nonsignificant increase compared to controls
- No differences among groups in pregnancy or fertility indices
- Lower mating indices, compared to controls, for females but not males, in groups III and VI; no effect on mating index in other groups
- Variation between groups in time-to-mating could not be attributed to treatment

Table B.1. Terminal Maternal Body Weight and Organ Weights (grams ± S.D.), Teratology Study

Group/ppm	N	Final body weight	liver weight (ratio of liver weight to corrected body weight)	kidney weight (ratio of kidney weight to corrected body weight)
I/0	18	357.0 ± 19.8	16.0 ± 1.5 (4.48)	2.0 ± 0.2 (5.57)
IV/500	10	377.6 ± 21.2*	17.1 ± 1.3 (4.52)	2.2 ± 0.2* (5.81)

*p <0.05

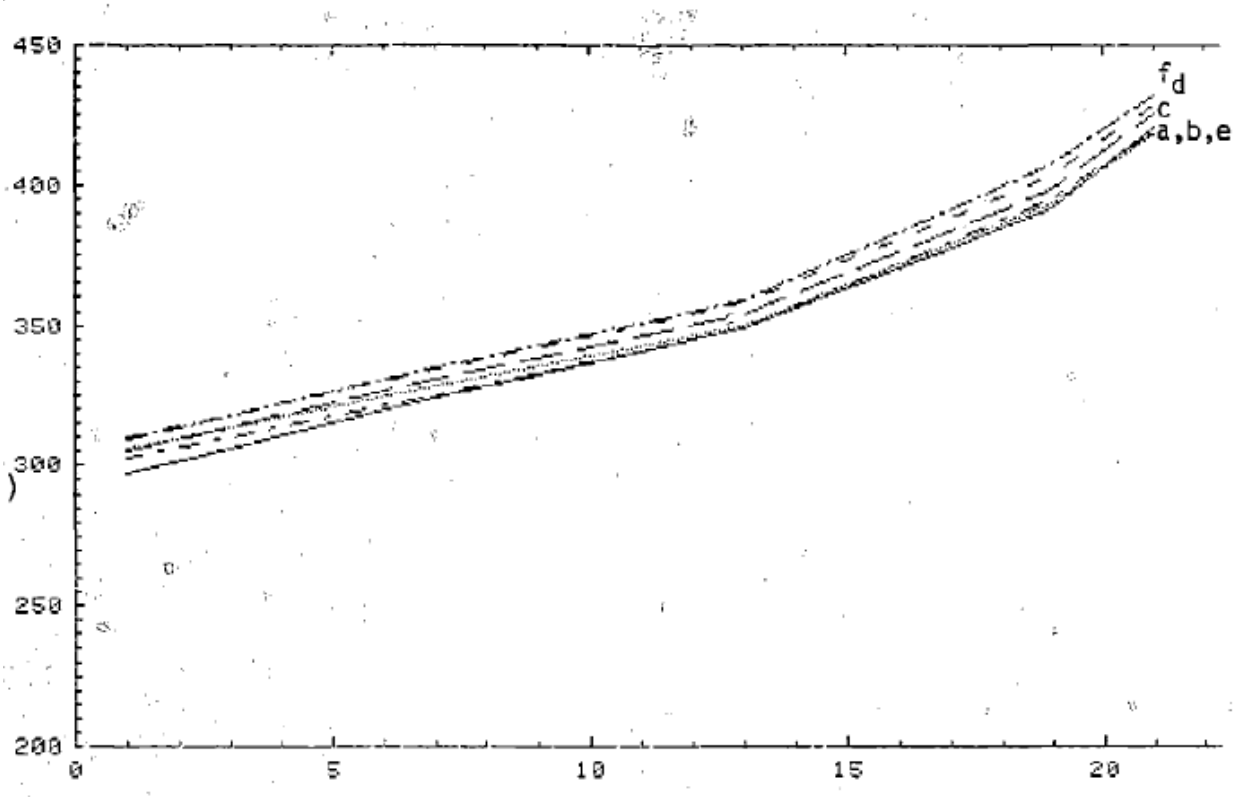
Table B.2. Mating Indices for Male and Female CD Rats

Group/ppm	Females Mated/Total Females		Males Mated/Total Males	
	Number	Percent	Number	Percent
I/0	59/59	100	29/29	100
II/60	19/20	95	9/10	90
III/250	17/20*	85	8/10	80
IV/500	36/40	90	19/20	95
V/500 (♂ only)	19/20	95	10/10	100
VI/500 (♀ only)	17/20*	85	9/10	90

* p <0.05

Figure B.1. Gestation Body Weights of Females Exposed to Mixed Xylenes

(Body Weight in Grams x Gestation Day)



a = group I, 0 ppm
b = group II, 60 ppm
c = group III, 250 ppm
d = group IV, 500 ppm
e = groupV, 500 ppm (♂ only)
f = group VI, 500 ppm (♀ only)

Results for teratology part of the study (control and 500 ppm groups) were as follows:

- No differences between groups for the number of dams with implantations, corpora lutea per litter, implantations per litter, resorptions per litter, or live fetuses per litter
- No effect on sex ratio
- No effect on body weight of male fetuses
- Significant decrease in weight of female fetuses exposed to 500 ppm xylene ($p < 0.05$)
- No evidence for a significant effect of treatment on the frequencies of external, internal, or skeletal variations or malformations

Table B.3. Litter Data for Animals Exposed to Mixed Xylenes (Teratology Part of the Study)

Group/ppm	N ♀ With implantations/N ♀	Corpora lutea/litter#	Implantations/litter#	Resorptions/litter#	Live fetuses/litter#
I/0	18/20	15.1	13.3	1.2	12.2
IV/500	10/12	15.6	12.6	1.6	11.0

Document did not provide S.D.s or S.E.s for these data

Table B.4. Fetal Weight and Sex Ratio for Animals Exposed to Mixed Xylenes (Teratology Part of the Study)

Group/ppm	Mean fetal weights (g) #		Mean number of fetuses per litter #		Fetal sex ratio (♂/♀)
	♂	♀	♂	♀	
I/0	3.57	3.51	6.4	5.7	1.13
IV/500	3.48	3.25*	5.4	5.6	0.96

* $p < 0.05$; # Document did not provide S.D.s or S.E.s for these data

Results for reproductive and developmental endpoints from the reproductive toxicity part of the study were as follows:

- No effect on gestation length or parturition
- No change in sex ratio, at birth or later time points
- According to the text, there were no effects on litter size or pup survival.
    - In Table B.5 below, however, data from the reproductive part of the study show that mean litter size for controls was low (9.6 pups per litter). Therefore, groups III and IV had significantly ($p < 0.05$) larger litters than

controls (12.5 and 12.4, respectively). The reduced litter size for controls may also have affected the findings for fetal weights.

  - In table B.7 below, the data show no effect on pup survival to PND 4. For survival from PND 4 to PND 21, however, 100% of pups in groups III (250 ppm) and V (500 ppm, ♂ only) survived. This was significantly greater than the 95.1% of surviving controls.

- Group IV (high dose): lower mean pup weights on PND 14, 21, 49
- Group III and IV (mid- and high-dose groups): lower mean ovary weight F1 females at PND 21, but not PND 49
- No differences among groups on PND 21 at gross necropsies or histological evaluation
- No evidence for an effect on testes weights for F1 males on PND-21 or PND-49

Table B.5. Litter Data for Animals Exposed to Mixed Xylenes (Reproductive Part of the Study)

Group/ppm	Number of litters	Gestation length (days)#	Live pups per litter, PND 1#
I/0	35	22.2	9.6
II/60	17	22.1	11.8
III/250	13	22.1	12.5*
IV/500	23	22.1	12.4*
V/500 (♂ only)	17	22.2	10.8
VI/500 (♀ only)	16	22.4	11.8

Document did not provide S.D.s or S.E.s for these data
* $p < 0.05$

Table B.6. Pup Weight Data (g) for Animals Exposed to Mixed Xylenes (Reproductive Part of the Study) PND 1, 4, 14, and 21

Group/ppm	PND 1	PND 4		PND 14	PND 21
	—	Pre--fostering	Post-fostering	—	—
I/0	6.6	9.0	9.2	23.5	36.7
II/60	6.3	8.4	8.5*	22.9	35.0
III/250	6.1	8.5	8.5*	22.9	34.3
IV/500	6.2	8.3	8.4**	21.3	32.9*
V/500 (♂ only)	6.5	8.8	8.8	23.2	34.9
VI/500 (♀ only)	6.3	8.5	8.7	22.2	36.0

Document did not provide S.D.s or S.E.s for these data
* $p < 0.05$; ** $p < 0.01$

Table B.7. Viability Indices for F1 CD rats

Group/ppm	PND 1-4#		PND 4-21##	
	Number	Percent	Number	Percent
I/0	323/336	96.1	233/245	95.1
II/60	193/201	96.0	130/133	97.7
III/250	154/162	95.1	104/104*	100
IV/500	279/286	97.6	182/184	98.9
V/500 (♂ only)	181/183	98.9	135/135**	100
VI/500 (♀ only)	185/189	97.9	126/128	98.4

*p < 0.05; **p < 0.01
#Before culling/cross-fostering on PND 4
##After culling/cross-fostering on PND 4

Table B.8. Body Weight and Gonad Weight Data F1 CD rats, PND 21 (Mean ± SD)

Group/ppm (N)	Body weight (g) all pups		Testes one ♂ pup/litter		Ovaries one ♀ pup/litter	
	Male	Female	Weight (g)	testis/bw	Weight (g)	ovary/bw
I/0 (N = 29♂ 31♀)	37.7 ± 5.9	36.8 ± 6.7	0.1531 ± 0.0279	4.06 ± 0.38	0.0087 ± 0.0025	2.38 ± 0.63
II/60 (N = 17♂ 17♀)	32.9 ± 6.0	33.6 ± 4.7	0.1327 ± 0.0322	3.99 ± 0.42	0.0073 ± 0.0027	2.15 ± 0.74
III/250 (N = 13♂ 13♀)	34.6 ± 6.3	35.1 ± 5.7	0.1467 ± 0.0337	4.22 ± 0.50	0.0066* ± 0.0018	1.91 ± 0.45
IV/500 (N = 23♂ 23♀)	35.9 ± 7.1	33.8 ± 5.1	0.1429 ± 0.3040	3.97 ± 0.39	0.0068* ± 0.0022	2.01 ± 0.59
V/500 (N = 17♂ 17♀)	36.1 ± 5.8	34.9 ± 5.4	0.1476 ± 0.304	4.09 ± 0.52	0.0069 ± 0.0023	1.99 ± 0.61
VI/500 (N = 23♂ 16♀)	35.3 ± 5.3	36.2 ± 6.6	0.1451 ± 0.0316	4.10 ± 0.57	0.0080 ± 0.0036	2.19 ± 0.82

*p <0.05;
Group V, treated only ♂
Group VI, treated only ♀

Table B.9. Body Weight and Gonad Weight Data F1 CD rats, PND 49

Group/ppm	Body weight (g) all pups		Testes one ♂ pup/litter		Ovaries one ♀ pup/litter	
	Male	Female	Weight (g)	testis/bw	Weight (g)	ovary/bw
I/0	220	160	2.045	0.91	0.055	3.42
II/60	215	158	1.984	0.90	0.059	3.67
III/250	212	163	1.912	0.93	0.065	3.88
IV/500	202*	149**	1.924	0.92	0.054	3.54
V/500 (♂ only)	214	162	1.877	0.87	0.053	3.30
VI/500 (♀ only)	212	160	1.946	0.90	0.057	3.61

N, S.D., or S.E. not provided for these data
*p < 0.05
**p < 0.01

Overall, the data from this study did not provide clear evidence for significant effects of xylene on reproductive function. Results for the teratology part of the study revealed no differences between control and exposed groups for the number of dams with implantations, corpora lutea per litter, implantations per litter, resorptions per litter, or live fetuses per litter. There was no effect on sex ratio, and no effect on the body weight of male fetuses. This study provided no evidence for a significant effect of treatment on the frequencies of external, internal, or skeletal variations or malformations. The only significant effect observed was a decrease in the weight of female fetuses exposed to 500 ppm xylene.

Among the litters allowed to go to term, mean litter size for the controls was only 9.6 pups per litter. Therefore, groups III and IV (60 and 250 ppm, respectively), had significantly larger litters than controls (12.5 and 12.4 pups, respectively). The reduced litter size for controls may also have affected the findings for fetal weights, as fetuses/pups tend to be larger in smaller litters (US EPA, 1991). Nonetheless, mean pup weights on PND 1 did not differ among groups.

Hass and Jakobsen, 1993. Prenatal toxicity of xylene inhalation in the rat: a teratogenicity and postnatal study

Seventy-two timed-pregnant Wistar rats were randomly divided into two groups of 36 each. Inhalation chambers were used to expose the treated group to air containing 200 ppm technical xylene for six hours per day on gestation days 4-20. Control animals were similarly exposed to air alone. Food and water were withheld while animals were in the inhalation chambers. Animals were observed daily for symptoms of toxicity; body weights and food consumption were recorded on gestation days 3, 10, 17 and 21.

Endpoints and Results at Term Following Prenatal Exposure to Xylene
On gestation day 21, two thirds of the rats were sacrificed and their uterine contents evaluated for standard teratological criteria. Endpoints consisted of:

- gravid uterine weight

- numbers of corpora lutea, implantations, and live, dead, or resorbed fetuses
- weights of live fetuses
- gross external malformations on live fetuses
- half of fetuses examined for soft tissue anomalies, the other half for skeletal alterations

No clinical signs of maternal toxicity were observed during the exposure period. No differences were observed between the treated and control groups for maternal body weight during either gestation or lactation, nor did food consumption vary between groups. Uterine weights on gestation day 21 also did not differ between treated and control groups.

Reproduction and litter data did not reveal any significant differences between treated and control groups. Fetal viability, body weight, and sex distribution were all unaffected. Preimplantation loss was slightly increased in treated (10.2%), relative to control (6.0%), litters, but this difference was not statistically significant.

Incidences of visceral anomalies or malformations was similar between the two groups. Skeletal anomalies, such as ossification of the fontanelle or sternebrae, or the frequency of extra ribs and/or spur(s), did not vary with treatment. The xylene-exposed group, however, did show a large increase in ossification delays for bones of the skull other than the fontanelle ($p < 0.001$, man-Whitney *U*-test). Closer examination revealed that this was primarily the result of delayed ossification of the *os maxilliare* in treated animals (18/26 litters) as compared to controls (2/22 litters).

Postnatal Endpoints and Results Following Prenatal Exposure to Xylene

The remaining one third of the dams were allowed to deliver their litters normally. Following delivery, both the dams and the individual pups were weighed. All litters were culled to eight pups, preferably four males and four females. A single litter of only five pups was born two days later than the others, and was excluded from further testing. The expected day of delivery, gestation day 22, was considered to be postnatal day zero for the pups.

In this part of the study, no differences were seen between treated and control groups in gestation length, number of pups per litter, or sex ratio. Male birthweights and pup weights for both sexes on postnatal day 28 were significantly higher than controls in the xylene-exposed group ($p < 0.05$, ANOVA).

Table B.11. Birth Data and Postnatal Weights

Endpoint	0 ppm xylene	200 ppm xylene
Number litters	10	10 (9)#
Gestation length	22.4 ± 0.3	22.5 ± 0.3
Pups per litter	8.6 ± 2.6	9.5 ± 0.8
% male pups	38%	47%
Birthweight, males*	6.1 ± 0.4	6.6 ± 0.4**
Birthweight, females*	6.1 ± 0.5	6.2 ± 0.4
Pup weight, PND 14, males*	27 ± 1.9	28 ± 1.1
Pup weight, PND 14, females*	27 ± 1.4	27 ± 1.4
Pup weight, PND 28, males*	73 ± 6	82 ± 4**
Pup weight, PND28, females*	71 ± 4	76 ± 2**

#One litter of five pups excluded from further analysis
*Weight in grams
**p <0.05, ANOVA

Pups were evaluated for physical and behavioral development in a postnatal test battery as described below:

- Body weights of both offspring and dams were taken on postnatal days 7, 14, 21, and 28
- Pinna unfolding, from PND 3
- Surface righting, from PND 3
- Cliff avoidance reflex, PND 7
- Incisor eruption, from PND 9
- Auditory startle, from PND 13
- Eye opening, from PND 13
- Air righting, from PND 15
- Rotarod, PND 22, 23, 24

Data for developmental milestones indicated that exposed pups were, if anything, slightly more advanced compared to the control group, but these differences were generally not statistically significant. Significant differences were seen only for ear unfolding and eye-opening (p <0 .05, ANOVA).

Table B.12. Day of Acquisition of Developmental Landmarks

Endpoint	0 ppm	200 ppm
Number litters	10	9
Pinna unfolding, males	3.6 ± 0.4	3.1 ± 0.2*
Pinna unfolding, females	3.3 ± 0.3	3.0 ± 0.1*
Eye-opening, males	15.2 ± 0.3	14.6 ± 0.5*
Eye-opening, females	15.2 ± 0.4	14.6 ± 0.4*

*p <0.05, ANOVA

Rotarod time was reduced for exposed female pups on all three test days (22-24), but only the reductions seen on PND 22 and 23 were statistically significant (p <0.05 or 0.01). For males, Rotarod time was significantly reduced (p <0.01) only on the second test day (23).

Table B.13. Rotarod Performance (seconds, mean ± SD)

Rotarod, day of testing, sex	0 ppm (10 litters)	200 ppm (9 litters)
PND 22, males	12 ± 5	15 ± 5
PND 22, females	13 ± 2	10 ± 4*
PND 23, males	26 ± 3	21 ± 4**
PND 23, females	24 ± 3	16 ± 5**
PND 24, males	23 ± 3	25 ± 4
PND 24, females	26 ± 3	22 ± 4#

#p <0.10, *p <0.05, **p <0.01, Mann-Whitney *U*-test

In their conclusions, the authors note the difficulty in interpreting their data on delayed ossification of the *os maxillare*, as other ossification delays were not observed. They suggest that the data could be interpreted as indicating that exposure to 200 ppm xylene is not a true no-effect-level for teratological effects.

The authors also discuss the postnatal increases in mean body weights for exposed pups relative to control pups. They note that parameters such as gestation length and litter size did not differ between groups in this study, and therefore do not explain the body weight differences. According to the authors, other organic solvents (i.e. toluene) have been associated with similar findings. The higher body weights for exposed pups were, in turn, taken as a likely explanation for the advanced development of some physical milestones in the same group.

Exposure-related impairment of Rotarod performance, which was mainly significant in females, could be interpreted as an indicator of impaired motor ability. Alternative influences could include the motivation of the animals and experimenter expectations — which the authors raise as a possible results of the behavioral tests not having been done blind to exposure group.

Hass et al., 1995. Effects of prenatal exposure to xylene on postnatal development and behavior in rats

Timed-pregnant rats (Mol:WIST) were exposed to 500 ppm technical xylene for six hrs/day on gestation days 7-20. This concentration and exposure period were specifically selected to avoid both maternal and embryo-fetal frank toxicity. Exposure was to a technical xylene consisting of: 19% o-, 45% m-, 20% p-xylene, and 15% ethylbenzene.

Food and water were withheld during the exposure period, and returned less than one hour following the end of exposure for the day. Daily observations were made to assess any clinical signs of toxicity. Body weight and food consumption were recorded on the mornings of gestation days 0, 6, 10, 15, and 20. Following exposure on gestation day 20, dams were housed alone with nesting materials.

Once parturition was completed, pups were counted, sexed, and checked for external anomalies. Dams and pups were weighed. Any dead pups found were examined. Litters were not culled to a standard size, but litters of less than six pups were excluded from further evaluation. PND 0 was defined as GD 22, rather than as the day of birth, per se.

Endpoints and Results at Birth Following Prenatal Exposure to Xylene
Pregnancy data are presented in Table B.10 below. A total of 13 control and 15 exposed litters were evaluated. No evidence was found for maternal toxicity, or for adverse impacts of exposure on gestation. Neonatal viability and sex ratio were not affected by prenatal exposure to mixed xylene at 500 ppm. According to the text of the paper, mean birth weight for exposed litters was reduced by approximately 5% relative to controls, but the difference was not statistically significant.

Table B.10. Pregnancy and Litter Data

Endpoint	Control	500 ppm Xylene
Number of litters	13	15
Maternal gestational weight gain (g)	90 ± 11	84 ± 23
Gestation length (days)	22.3 ± 0.3	22.3 ± 0.6
Implantations/litter	12.6 ± 1.8	11.7 ± 2.7
Live pups/litter, PND 1	11.7 ± 2.1	11.4 ± 2.7
Neonatal deaths/litter	0.2 ± 0.4	0.5 ± 0.9
% Male pups	47 ± 13	44 ± 13
Mean birth weight (g)	6.3 ± 0.5	5.9 ± 0.5

Postnatal Endpoints and Results Following Prenatal Exposure to Xylene

Pups were evaluated in a postnatal test battery as described below:

- Pre-weaning
    - Pinna unfolding, all pups, from PND 2
    - Surface righting, all pups, from PND 2
    - Homing response, all pups, PND 6-7
    - Incisor eruption, all pups, from PND 10
    - Auditory startle, all pups, from PND 12
    - Eye opening, all pups, from PND 13
    - Air righting, all pups, from PND 15
- Post-weaning
    - Rotarod, 1 pup/sex/litter, PND 24-26
    - Open field, 1 pup/sex/litter, PND 27 and 34 (± 2)
    - Sexual maturation, 1 pup/sex/litter, from PND 30
    - Morris water maze, 2 pups/sex/litter, 3-4 months (1 pair/litter were "standard-housed" and 1 pair/litter were "enriched-housed")

Following weaning on PND 22, two males and two females of median body weights from each litter were kept for further testing. One set from each litter were "standard-housed" in small rats cages paired with cage mates of the same sex and same prenatal exposure. These animals were left undisturbed apart from feeding and recording of bodyweights (once every second week) until the age of 3 months. Testing in the Morris water maze took place at that time.

The other male and female from each litter were placed into what was called "enriched-housing." This consisted of group housing 4-5 animals of the same sex and same exposure in large cages with toys available. The enriched-housed pups were tested on Rotarod and Open Field, as described above, and eventually in the Morris water maze. The technique for administration of the Morris water maze test is described in detail in the summary of Hass et al., 1995, below.

Remaining pups, dams, and other sperm-positive females were sacrificed on PND 28 for physical examinations. Brain weights were recorded for at least one randomly-selected pup of each sex per litter.

Behavioral tests were performed during the animals' dark period, when they are most active. Apart from the litter and pregnancy data summarized above (Table B.10), results were as follows:

- Brain weights of male and female rats on PND 28 were not significantly affected by prenatal exposure to 500 ppm xylene.
- Body weights did not show significant differences between groups, during either the pre-weaning or the post-weaning periods.

- The air righting reflex was significantly delayed (Mann-Whitney *U*-test: $p < 0.05$) with exposure to xylene, for litters tested on PND 15 and 16. When considered separately, treated males and females were both delayed relative to their own controls, but only the data for females showed statistical significance (Mann-Whitney *U*-test: $p < 0.05$). By PND 17 all pups were able to air-right.
- Other developmental landmarks were not affected by prenatal xylene exposure.
- The Rotarod test did not detect statistically significant differences between treated and control groups on any of the testing days. The trend, however, was for a greater percentage of exposed animals to fail at reaching 30 seconds on the rod, and to have a shorter mean time on the Rotarod. This trend was most marked in female offspring in the third trial on PND 26 (Mann-Whitney *U*-test: $p=0.077$).
- The Open Field test revealed no exposure-related differences between treated and control groups for ambulation, the percent of time spent in the center of the field, or the frequency of defecation.
- Water maze performance was initially influenced by the type of housing, so the two groups were analyzed separately.
    - No exposure-based differences were found for the enriched-housed animals.
    - For the standard-housed offspring, a non-significant trend suggested that xylene-exposed animals took slightly longer to find the platform during initial test blocks (F (1, 25) = 3.928, $p = 0.059$).
    - No exposure-related differences between groups were seen for memory after three weeks, or during reversal learning when the platform was moved to the opposite quadrant of the pool.
- Subsequent water maze experiments appear to focus on standard-housed animals, as enriched-housed animals were not affected.
    - Exposed offspring took significantly longer to find the platform following its relocation to the center of the pool (F (1, 26) = 7.613, $p = 0.010$).
    - Separate analysis of each sex revealed that the exposure-based difference was significant only for female offspring (F (1, 25) = 8.998, $p = 0.006$). Exposed females also showed a significant increase in swimming time (F (1, 23) = 5.298, $p = 0.031$), but no difference in swimming speed relative to controls.

The authors concluded that their study detected postnatal effects of prenatal exposure to xylene, which were particularly marked in female offspring. While other developmental landmarks were unaffected, acquisition of the air righting reflex was delayed in exposed pups. These findings were taken to suggest damage to neural processes required for air righting — such as vestibular function — rather than a more general developmental delay.

Results of the Rotarod performance tests were taken to indicate a trend toward impairment of motor coordination and balancing abilities in exposed female offspring. The authors cite other studies as showing a link between performance deficits in the Morris water maze and hippocampal dysfunction in adult rats.

According to the authors, gender differences in offspring behavior are not unusual in behavioral teratology studies. The impairment in water maze performance was seen only in the standard-housed but not in the enriched-housed offspring, suggesting that the effect of prenatal xylene was at least partially overcome by the enriched housing environment.

Overall, the authors conclude that at least some of the observed alterations may be irreversible, as they were identified in adult rats following prenatal exposure.

Hass et al., 1997. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats

As a follow-up to their previous study (Hass et al., 1995), an additional investigation was conducted specifically to look at impaired performance in the Morris water maze test by young female rats exposed in utero to xylene. The treatment protocol was as described in the previous study:

- Timed-pregnant rats (Mol:WIST) were exposed to 0 or 500 ppm technical xylene for six hrs/day on gestation days 7-20.
- Exposure was to a technical xylene consisting of: 19% o-, 45% m-, 20% p-xylene, and 15% ethylbenzene.
- Food and water were withheld during the exposure period, and returned less than one hour following the end of exposure for the day.
- Daily observations were made to assess any clinical signs of toxicity.
- Body weight and food consumption were recorded on the mornings of gestation days 0, 6, 10, 15, and 20.
- Following exposure on gestation day 20, dams were housed alone with nesting materials and left to deliver their litters normally.

The treatment regimen was designed to avoid both maternal and frank embryo-fetal toxicity. Neither maternal toxicity nor evidence of embryo-fetal toxicity was observed at birth. Offspring body weights did not differ between treated and control animals during the entire study period.

Once the delivered pups were weaned, one female pup per litter, selected as having the median body weight, was reserved for further investigations. These pups were given standard food and water ad lib, weighed every other week, and otherwise left undisturbed until they reached three months of age when testing commenced.

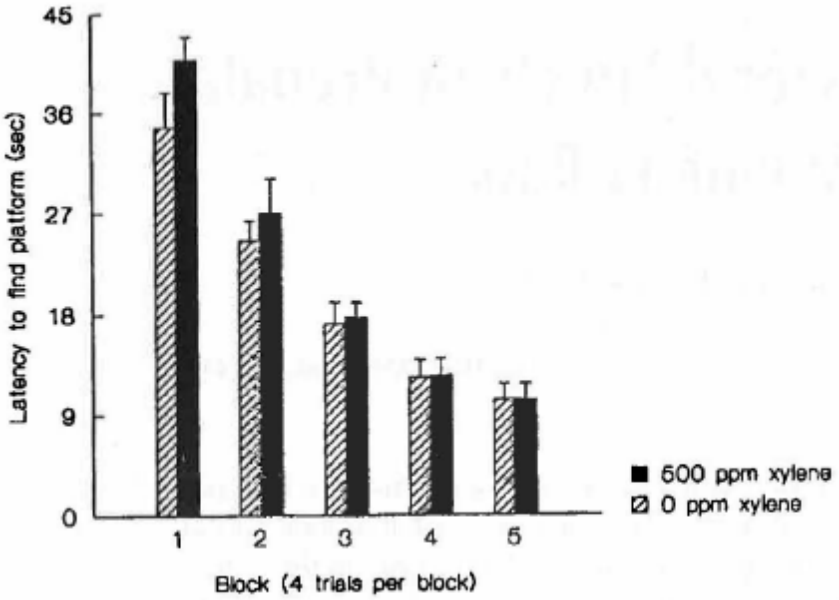
The Morris water maze is a test of spatial navigation in which rats are required to locate a small platform under the surface of the water. The animals were tested at the ages of 12-16 weeks, 28 weeks, and 55 weeks. Testing was performed during the animals' dark period, when they are normally most active.

- The maze was a round, black plastic pool, 40 cm deep and 100 m in diameter.

- The water was 30 cm deep, and kept at room temperature (22°C).
- The pool was divided into quadrants by using compass designations (N, S, E, W) to mark points on the rim of the pool.
- A 10 cm circular platform was located 1 cm below the surface of the water
- The female rats were tested in blocks of four trials, using each of the four compass points as starting positions "assigned in a pseudo-random sequence."
- The trial ended when the rat swam to and climbed on to the platform, or when 60 seconds had elapsed.
- Animals failing to find the platform within 60 seconds were placed on the platform and left for 15 seconds.
- Video tracking was used to electronically calculate path lengths and swim speeds.
- An experimental scheme for altering positions of the platform in subsequent trials was used to test for aspects of learning (including reversal learning and transfer of learning) and memory.

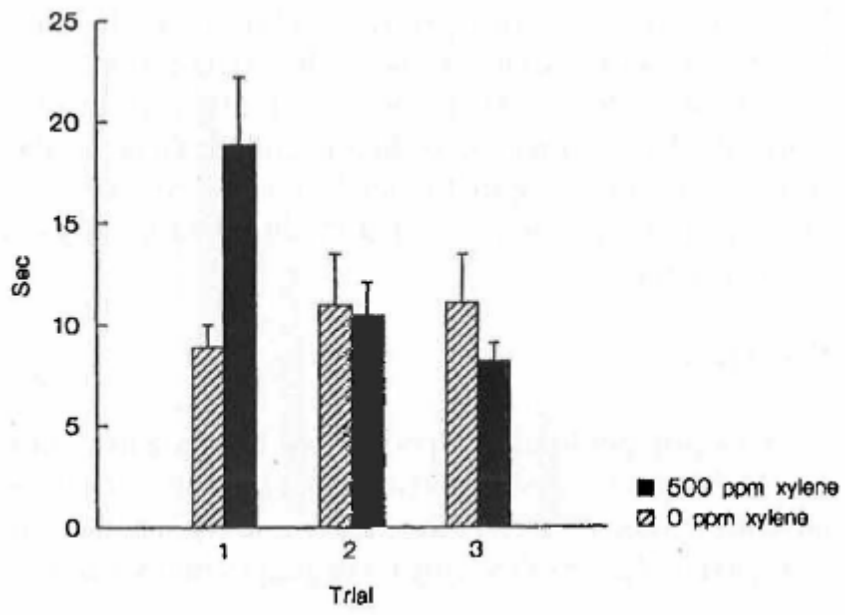
When tested initially at week 12, the xylene-exposed offspring took significantly longer than control offspring to find the platform ($p = 0.059$). When the test was repeated four weeks later (16 weeks postnatal age), no difference was observed between groups whether the platform within the same position as before (memory test) or had been moved to the opposite quadrant of the pool (reversal learning). Differences between treated and control animals were revealed when the platform was moved to the center of the pool, with exposed offspring taking significantly longer to find the relocated platform ($p = 0.031$). Further analysis indicated that swim speed was not different between the treated and control groups, and hence did not account for the increased latency. The xylene-exposed group showed a significantly increased length of swim path ($p = 0.031$).

Figure B.2. Initial Latency in 12-Week Old Mice to Find the Hidden Platform in the Morris Water Maze (Mean ± SEM; N = 13-17)



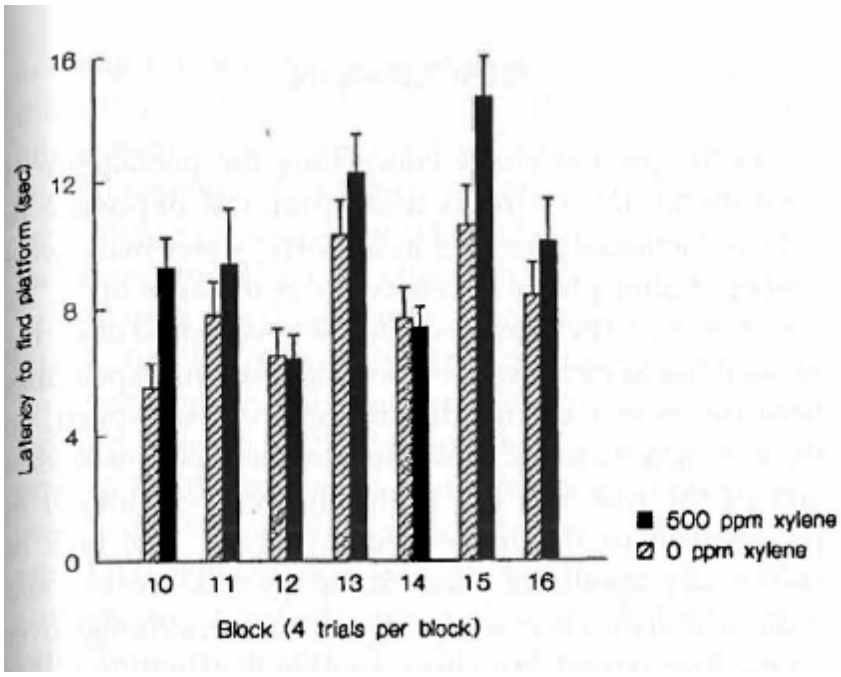
When tested at the age of 28 weeks, control and treated groups did not differ significantly in water maze performance when the platform was placed either in its initial position, or in the center of the pool. In other words, the animals had previous experience with these platform placements. When the platform was moved to a new position at the rim of the pool, increased latency was observed in xylene-exposed animals. This latency was significant in the first trial of the block ($p = 0.013$). When this result was broken out into consideration of swimming path length versus swimming speed, swimming path length was found to be significantly increased ($p = 0.014$). Swim speed did not vary between groups.

Figure B.3. Initial Latency in 28 Old Mice to Find the Hidden Platform in the Morris Water Maze (Mean ± SEM; N = 13-17)



At 55 weeks of age, treated and control groups did not differ in their ability to find the platform located in what had been the new position at 28 weeks. Treated animals did show a greater, but not statistically significant, latency in finding the platform at earlier test positions (i.e. those used at 12 weeks).

Figure B.4. Summary Chart of Experimental Conditions Showing Differences between Treated and Control Groups (Mean ± SEM; N = 13-17)



block 10: page 16 weeks, platform relocated to center of pool (p = 0.006)
block 11: age 28 weeks, platform in SW quadrant of pool
block 12: age 28 weeks, platform relocated to center of pool
block 13: age 28 weeks, platform relocated at rim of pool (p = 0.013 in first trial of block)
block 14: age 55 weeks, platform in SW quadrant of pool
block 15: age 55 weeks, platform relocated to NE quadrant of pool
block 16: age 55 weeks, platform relocated to center of pool

In summary, increased latencies in the Morris water maze were observed in animals prenatally exposed to xylene when tested at postnatal ages of 16, 28, and 55 weeks. These differences did not appear to be related to swim speed or other motor difficulties with swimming. On the other hand, swim path lengths of treated animals were longer in proportion to the increased latencies. By 55 weeks of age, effects were not statistically significant, suggesting at least partial reversibility of xylene-induced impairment after a long period of time. Alternatively, repeated testing may have allowed the animals to develop skills at locating the platform, helping to compensate for any organic deficits caused by prenatal xylene exposure.

Hudak and Ungvary, 1978. Embryotoxic effects of benzene and its methyl derivative: toluene, xylene

Pregnant CFY rats were exposed to 0 or 1,000 mg/m^3 (230 ppm) xylene for 24 hrs/day, from GD 9-14. Untreated controls, as well as controls exposed only to air in the inhalation chambers were used. Animals were sacrificed on GD 21, and their term fetuses removed for evaluation.

No significant differences were found between untreated control animals and those controls exposed to air in the inhalation apparatus. Exposure to 230 ppm xylene, continuously on gestation days 9-14, caused no maternal mortality and had no effect on maternal gestational weight gain. Treatment had no significant effect on fetal loss or mean litter size, or on mean fetal or placental weights.

Table B.14. Litter Data for Pregnant Rats Exposed to 230 ppm Xylene During Pregnancy

Group	N pregnant ♀/group	Maternal weight gain (% initial bw, ± SE)	Fetal loss (% of implants)	Mean litter size (± SE)	Mean fetal weight (g, ± SE)	Mean placental weight (g, ± SE)
Untreated control	28	52.45 ± 1.23	3.37	11.25 ± 0.54	3.83 ± 0.02	0.51 ± 0.005
Air control	26	46.86 ± 2.04	4.13	13.38 ± 0.50	3.76 ± 0.02	0.47 ± 0.004
230 ppm xylene	20	46.20 ± 2.01	5.30	14.30 ± 0.57	3.75 ± 0.02	0.51 ± 0.003

Agnathia, or missing mandible, was reported as the only external abnormalities observed in 2/286 xylene-exposed fetuses. This defect was not reported in either of the control groups. Dilated urinary tracts were observed in both control groups as well as in treated animals, and were not considered to vary significantly among groups. Fused sternebrae and extra ribs were both significantly increased in xylene exposed animals ($p < 0.05$, for both endpoints). One xylene-exposed fetus was described as having fissura sterni, a defect that was not observed in any of the other groups.

Table B.15. Malformation and Skeletal Data for Fetuses Exposed to 230 ppm Xylene

Effects	Untreated controls	Air inhalation	Xylene, 230 ppm
N litters	28	26	20
N live fetuses	315	348	286
External malformations	—	—	—
Agnathia**	0	0	2
N fetuses dissected	166	179	146
Internal malformations	—	—	—
Hydronephrosis	3	16	26
N alizarin-stained fetuses	143	169	143
Exhibited signs of skeletal retardation*	0	11	17
Skeletal anomalies	—	—	—
Fused sternebrae	1	2	8 #
Extra ribs	2	0	9 #
Skeletal malformations	—	—	—
Fissura sterni	0	0	1
Agnathia**	—	—	2

*Poorly ossified sternebrae, bipartite vertebra centra, and/or shortened 13th ribs
**"Agnathia" is categorized both as an externally-visible malformation and as a skeletal malformation
#p <0.05 (Mann-Whitney U test)

It should be noted that anomalies do not appear to have been evaluated on a per litter basis in this study.

From the lack of differences between the untreated controls and the air-inhalation controls, the authors conclude that simply being kept in the inhalation chamber had no adverse effect on these animals. No explanation is offered for the nonsignificant increases in the frequencies of hydronephrosis and skeletal retardation among air-inhalation controls as compared to untreated controls. The authors suggest that xylene, among the other test solvents studied (data not presented here), might have caused an irritating effect on the dams. The resulting stress response might then be responsible for observed skeletal anomalies.

The authors conclude that their test solvents, which included benzene and toluene as well is xylene, all had embryotoxic effects.

Kükner et al., 1997. The Effect of Xylene Inhalation on the Rat Liver

Seven pregnant Wistar rats were exposed to xylene by inhalation at a concentration of 11284 mg/m^3 (2600 ppm) for eight hours per day, seven days per week, from gestation

day six through term. A control group of five pregnant animals were exposed only to clean air in the inhalation chambers. An additional group of five non-pregnant female rats were exposed to xylene under the same conditions as the test pregnant animals.

Xylene treatment did not affect gestational weight gain, or weight gain of the non-pregnant females. Blood samples from pregnant rats did not reveal significant differences among groups in hemoglobin and hematocrit values. Samples of maternal liver, however, showed xylene-treatment related increases in:

- Aspartate aminotransferase (AST) activity (18%↑)
- Alanine aminotransferase (ALT) activity (19%↑)
- Alkaline phosphatase (ALP) level (17%↑)
- Arginase activity (63%↑)

Six [treated] pregnant rats gave birth to a total of 39 pups. Newborn pups weighed an average of 5.7 g (range: 4.8-7.5 g). No external abnormalities or internal gross soft tissue anomalies were observed in these pups.

Tissue samples were taken from five newborn rat pups. Light and electron level microscopy revealed no structural changes in the pancreas or kidney tissues of rat pups prenatally exposed to xylene. Histology of their livers, however, showed a number of pathological changes:

- Disarrayed membranes of hepatocyte nuclei
- Heterochromatic nuclei
- Vacuolization
- Deformed, swollen, elongated hepatocyte mitochondria
- Dilated rough endoplasmic reticulum
- Dense primary and secondary lysosomes in the cytoplasm of Kupffer cells
- Hepatocyte cytoplasm lighter in color in treated pregnant and non-pregnant animals, as compared to controls
- Mitochondria concentrated at the periphery of hepatocytes and around the nucleus
- Increased number of lysosomes
- Numerous smooth and of plasma structures having a web-like appearance with glycogen particles

While no frank embryotoxic effects were observed with prenatal-xylene exposure, structural changes occurred in fetal liver tissue.

Litton Bionetics, 1978. Final Report: Teratology Study in Rats

Female CRL:COBS CD (SD) BR rats were obtained from Charles River Breeding Laboratories, Inc., and time-mated in-house. According to the protocol information provided, following confirmation of mating, females were assigned sequentially to

treatment groups. Treatment consisted of exposure to airborne concentrations of xylene at zero, 100, or 400 ppm, on each of gestation days 6-15. Effective daily exposure was six hours.

- Pregnant female rats weighed on GDs 0, 6, 15, and 20
- 26 animals per group
- Food consumption measured for GDs 0-6, 6-15, and 15-20
- Daily observations for clinical symptoms of toxicity
- Sacrifice for evaluation of uterine contents on GD 20
- Fetuses examined by standard methodologies
    - Removed, weighed, and examined for external abnormalities
    - One third of each litter fixed for soft tissue internal evaluation
    - Remaining two thirds were fixed, cleared, and stained for skeletal evaluations
- Empty uteri fixed and saved, but never evaluated

Maternal animals did not vary among groups for mean body weights or food consumption. Necropsies of these animals did not reveal gross visceral pathology. Nor were significant differences found among litters of different groups at fetal evaluation for parameters of viability, growth, or malformation frequency. Concurrent control and historical control data "were considered to be acceptably similar."

Table B.16. Litter Data for Pregnant Rats Exposed to Xylene GD 6-15

Endpoint	0 ppm	100 ppm	400 ppm
Live litters/bred	24/26	25/26	25/26
Litters with resorptions	8 (33%)	10 (40%)	13 (52%)
Litters with dead fetuses	0	1	0
Mean live litter size*	12	12	12
Mean fetal weight (g)*	3.5	3.5	3.4
Live fetuses/implantation sites	289/304 (95%)	289/306 (94%)	291/316 (92%)

*S.D. or S.E. not provided

Skeletal evaluations were considered to provide no evidence of treatment-related effects. A statistically significant increase in skeletal changes classified as "unusual" ($p < 0.05$) in the 400 ppm xylene group was seen only when evaluated for all fetuses, not on a per litter basis. The report offered no clear definition of what constituted an "unusual" skeletal observation. Although 10 litters in the 400 ppm xylene group were reported as having at least one affected fetus, the majority of affected fetuses were reported to have come from three specific litters. According to the authors, the fetuses from all three of these litters were considered to be small and developmentally retarded. The authors did not attribute these observations to the effect of xylene exposure.

Table B.17. Skeletal Changes in Offspring of Animals Treated with Xylene

Dose (ppm)	N fetuses examined (N litters)	N normal offspring	N offspring with "common" changes only (N litters)	N offspring with "unusual" skeletal variations (N litters)
0	196 (24)	123	53 (19)	19 (9)
100	197 (25)	115	58 (21)	24 (6)
400	201 (25)	119	45 (20)	37* (10)

*p <0.05

The authors concluded that exposure of pregnant rats to xylene at concentrations of 100 or 400 ppm had no effect on maternal animals. Nor did these exposure regimens produce changes in fetal sex ratio, viability, growth, or morphological development.

Marks et al., 1982. Teratogenicity of a commercial xylene mixture in the mouse.

Timed-pregnant CD 1 mice were exposed to a commercial mixture of xylene, given by gavage in three treatments per day, on each of gestation days 6-15. Cottonseed oil was used as the vehicle. Analysis of the xylene mixture determined its components as 17.0% ethylbenzene, 13.6% p-xylene, 60.2% m-xylene, 9.1% o-xylene, and less than 0.3% other volatile impurities.

Table B.18. Determination of Daily Xylene Doses From Marks et al., 1982

Xylene concentration in cottonseed oil (v/v)	0	2%	4%	8%	10%	12%	16%
Xylene dose (ml/kg-day)	0	0.6	1.2	2.4	3.0	3.6	4.8
Xylene dose* (mg/kg-day)	0	0.52	1.03	2.06	2.58	3.10	4.13

* density 0.86 g/ml

Pregnant females were assigned to each dose group "in such a way that body weight differences between the groups were minimized." The study was conducted as a series of five overlapping experiments, some of which were complete replicates. The experiments were designed as follows:

- Each replicate included a vehicle control.
- The first three replicates included the three lowest doses of 0.52, 1.03, and 2.06 mg/kg-day.
- The third experiment included the only appearance of the high dose, 4.13 mg/kg-day
- The fourth and fifth replicates included the two next highest doses of 2.58 and 3.10 mg/kg-day

Pregnant dams were sacrificed on gestation day 18, and their uterine contents were examined using standard teratological techniques.

None of the 15 pregnant dams exposed to the highest dose of 4.13 mg/kg-day survived until gestation day 18. Maternal mortality was 32% of 38 treated dams in the 3.10 dose group. All the pregnant females in all of the other dose groups survived the study. Other maternal endpoints included:

- Significantly decreased maternal gestational weight gain ($p < 0.05$) at the highest survivable dose of 3.10 mg/kg-day
- Significantly increased maternal liver weights ($p < 0.05$) at doses of 2.06 and 2.58 mg/kg-day
- Mean gravid uterine weights were significantly decreased ($p < 0.05$) at doses of 2.06, 2.58, and 3.10 mg/kg-day, in a dose-dependent manner

Litter endpoints included:

- No effect on implantation rate, sex ratio, or mean live litter size
- At 3.10 mg/kg-day, 13/20 litters were completely resorbed
- The frequency of resorptions out of total implants was 62.3% at 3.10 mg/kg-day, a finding that was statistically significant ($p < 0.05$)

Table B.19. Results from Marks et al., 1982

Endpoint/dose (mg/kg-day)*	**0**	**0.52**	**1.03**	**2.06**	**2.58**	**3.10**
Dams treated/dams survived	66/66	24/24	23/23	26/26	28/28	38/26
Pregnant dams	62	22	23	23	26	20
Maternal gestational weight gain (g)**	4.9±0.26	5.0±0.43	5.2±0.49	5.1±0.72	4.4±0.47	2.4±0.76 #
Gravid uterine weight (g)**	15.6±0.60	14.1±1.06	15.1±0.94	13.2±1.16 #	11.6±1.06 #	5.3±1.51 #
Maternal liver weight (g)**	2.2±0.03	2.2±0.57	2.3±0.05	2.4±0.08#	2.6±0.07#	2.3± 0.11
Total litter resorptions (total pregnant dams)	3 (62)	1 (22)	1 (23)	3 (23)	4 (26)	13 (20)
Resorptions as % of total implants**	11.2	14.7	16.2	19.2	20.3	62.3#
Mean live litter size±	11.2	9.9	10.5	11.4	10.2	11.3
Mean fetal weight per litter±**	0.982± 0.010	0.982± 0.014	0.975± 0.014	0.861± 0.021#	0.785± 0.017#	0.708± 0.024#

*The highest dose of 4.13 mg/kg-day is not included, as all dams died before the end of the study
**Significant trend (p <0.05) Jonckheere's test
#p <0.05, two-sided Mann-Whitney *U*-test
± Excludes totally resorbed litters

Fetal endpoints included:

- Significantly reduced mean fetal weights (p <0.05) at the three highest doses of 2.06, 2.58, and 3.10 mg/kg-day, in a dose-dependent manner
- Live fetuses weighing 0.5 g or less, or less than two thirds of the mean weight of their litter mates, were designated as stunted. Stunted fetuses were not scored for malformations. There were no significant differences among groups in the numbers of stunted fetuses
- One control fetus had meningoencephalocele

- Cleft palate was the most common malformations seen in other groups, with 1, 6, 11, and 7 cases seen in the 1.03, 2.06, 2.58, and 3.10 mg/kg-day dose groups, respectively
- Bilateral open eye and exencephaly were seen in the 2.06 mg/kg-day dose group
- Approximately 1/3 of the fetuses from each group were examined for visceral malformations; no visceral malformations were observed in any group; findings of cream-colored liver lobes and one fetus, and a white spot in the left ventricle of the heart in another fetus were not scored as malformations
- All fetuses were prepared for skeletal evaluations; low frequencies of skeletal malformations were scattered among the dose groups with no clear dose response effect
- Skeletal findings included fused [vertebral] arches and ribs, fused sternebrae, missing [vertebral] arches and ribs
- The frequency of total malformations, expressed as a mean percentage, was statistically significant (p <0.01) at doses of 2.06, 2.58, and 3.10 mg/kg-day, whether or not cases of bilateral wavy ribs were included
- Total malformations showed a significant trend (p <0.001) to increase in frequency with increasing dose

Table B.20. Effects of Xylene on the Frequency of Malformations in Mouse Fetuses

Endpoint/dose (mg/kg-day)*	**0**	**0.52**	**1.03**	**2.06**	**2.58**	**3.10**
Number fetuses/number litters	658/62	208/22	232/23	227/23	224/26	79/20
Fetuses with external malformations (litters containing malformed fetuses)	1 (1)	0 (0)	1 (1)	7 (5)	11 (5)	7 (4)
Fetuses with skeletal malformations (litters containing fetuses with skeletal malformations)	1 (1)	2 (2)	2 (2)	0 (0)	1 (1)	0 (0)
Total malformed fetuses (total litters with malformed fetuses)	2 (2)	2 (2)	3 (3)	7 (5)	12 (6)	7 (4)
Mean percent malformed fetuses excluding bilateral wavy ribs*#	0.3	1.0	1.0	3.4^	7.8^	9.1^
Mean percent malformed fetuses including bilateral wavy ribs*#	0.3	1.0	1.0	3.4^	10.5^	13.4^

*100 X ∑ (no. malformed fetuses/total no. litters)/total no. fetuses in litter
#Significant trend (p <0.001) with increasing dose (Jonckheere's test)
^ Two-sided p <0.01 versus vehicle control (Mann-Whitney U-test)

The authors conclude that xylene at doses of 2.06, 2.58, and 3.10 mg/kg-day caused a significant increase in the average percentage of malformed fetuses as compared to

vehicle controls. Maternal viability and gestational weight gain were only affected at the highest dose of 3.10 mg/kg-day.

The two lower doses of 2.06 and 2.58 mg/kg-day xylene were associated with significantly increased mean maternal liver weights. However, the authors note that they routinely weighed maternal livers as an indicator of maternal toxicity, but had been unable to correlate statistically significant changes in maternal liver weight with fetal effects.

Mirkova et al., 1979. Xylene neurotoxicity in pregnant rats and fetuses

The effects of dermal exposure to xylene were evaluated using 80 pregnant Wistar rats and their offspring. Applied doses were 0, 100, 200, and 2000 mg/kg-bw, daily throughout pregnancy (gestation day 1-20).

Pregnant dams were evaluated for open field behavioral activity on days 18-20 of gestation. At term, maternal and fetal brains were evaluated for:

- ChE and CytO activities (cholinesterase and cytochrome oxidase, respectively)
- Activities of the dehydrogenases LHD, MHD, i-CDH, G_6PDH, and SucDH (lactate dehydrogenase, [probably MDH, and therefore malate dehydrogenase], isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, succinate dehydrogenase)
- DNA concentration
- Soluble protein content

Results were analyzed by the Student "t" test.

Results from the open field test showed significantly reduced motor activity in pregnant females exposed to 2000 mg/kg xylene. The number of defecations while in the open field test was taken as an index of "emotionality," and was also significantly affected by exposure to the high dose of xylene. It is not clear from the paper whether this was an increase or decrease in the number of defecations, relative to controls. Nonetheless, these changes were taken as confirmation of xylene-induced neurotoxicity in the maternal animals.

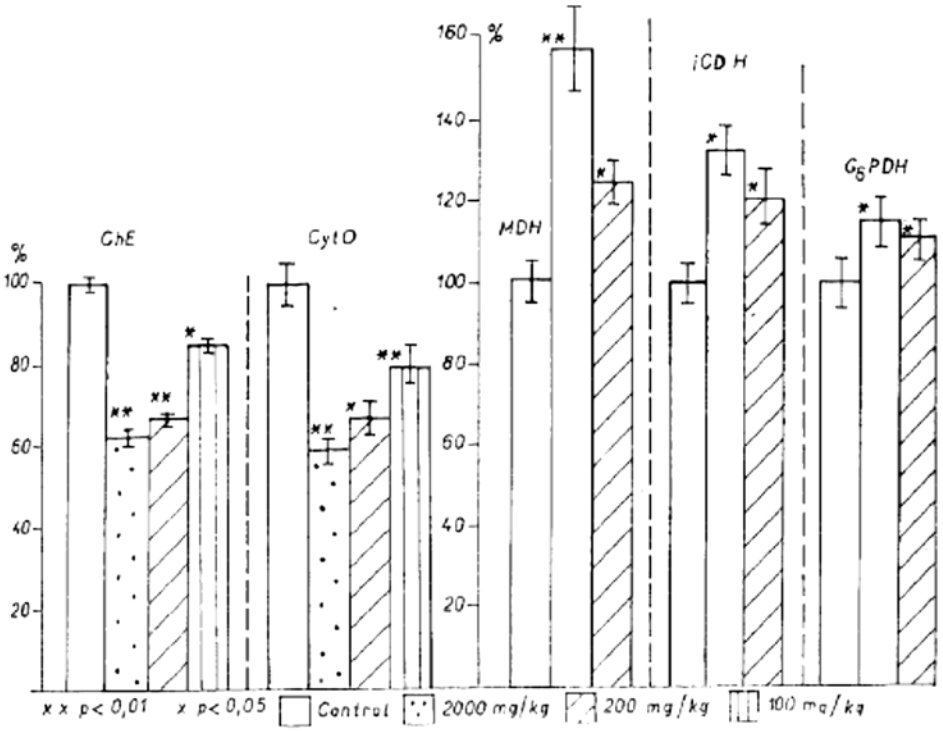


Figure B.5. Brain enzymes in pregnant rats and fetuses treated with xylene

According to the text of the paper, maternal brain cholinesterase (ChE) and cytochrome oxidase (CytO) activities were significantly decreased in a dose-dependent manner. The highest dose of xylene, 2000 mg/kg, was associated with increased glucose-6-phosphate dehydrogenase (G_6PDH) activity. The text also states that DNA concentrations and soluble protein content were reduced at 2000 and 200 mg/kg xylene.

Biochemical studies of fetal brains were stated to have found that ChE and CytO activities in fetal brains were inhibited at the 2000 and 200 mg/kg doses. At the same doses, fetal brain showed increases in the activities of MHD [MDH], isocitrate dehydrogenase, and G_6PDH.

Unfortunately, this study is poorly reported with regards to both methodology and results. Figure B.5. above does not clarify exactly which parts of the biochemical data presented apply to the maternal animals, and which to fetal tissues. Possibly both maternal and fetal results are incorporated into the results for each endpoint at each dose. Taken at face value, however, this study does suggest that xylene can be absorbed by the dermal route and may measurably affect fetal brains and potentially other tissues.

Mirkova et al., 1983. Prenatal Toxicity of Xylene

Pregnant Wistar rats were exposed by inhalation to xylene at concentrations of 0, 10, 50, or 500 mg/m^3 (0, 2.31, 11.53, or 115 ppm), for 6 hours per day, five days per week, from GD 1-21. On GD 21 some of the animals were sacrificed for fetal evaluation, while others were allowed to deliver their litters normally. Pups were evaluated at one month and three months postnatal age.

The paper makes no mention of maternal mortality or weight gain during the study. Litter and fetal variables from the teratology part of the study were as follows:

- No effect of xylene treatment on gestation index, numbers of corpora lutea per litter, mean implantations per litter, mean live fetuses per litter, or resorption frequency per litter
- Post-implantation loss was significantly increased at 11.53 and 115 ppm xylene ($p < 0.05$ at both concentrations)
- Mean fetal weight per litter was significantly decreased at 11.53 and 115 ppm xylene
  ($p < 0.01$ and $p < 0.05$, respectively)
- The percentage of total fetuses with hemorrhages was significantly increased at 11.53 and 115 ppm ($p < 0.01$ at both concentrations); no data were provided on the numbers of litters with affected fetuses
- According to the text of the paper, the high concentration of 115 ppm xylene was associated with a significant increase in the incidence of visceral abnormalities, but data were not provided
- The text states that exposure to 11.53 or 115 ppm xylene was associated with significant increases in the frequency of skeletal anomalies of 62 and 177%, respectively

Table B.21. Litter Data for Wistar Rats Exposed to Xylene on GD 1-21

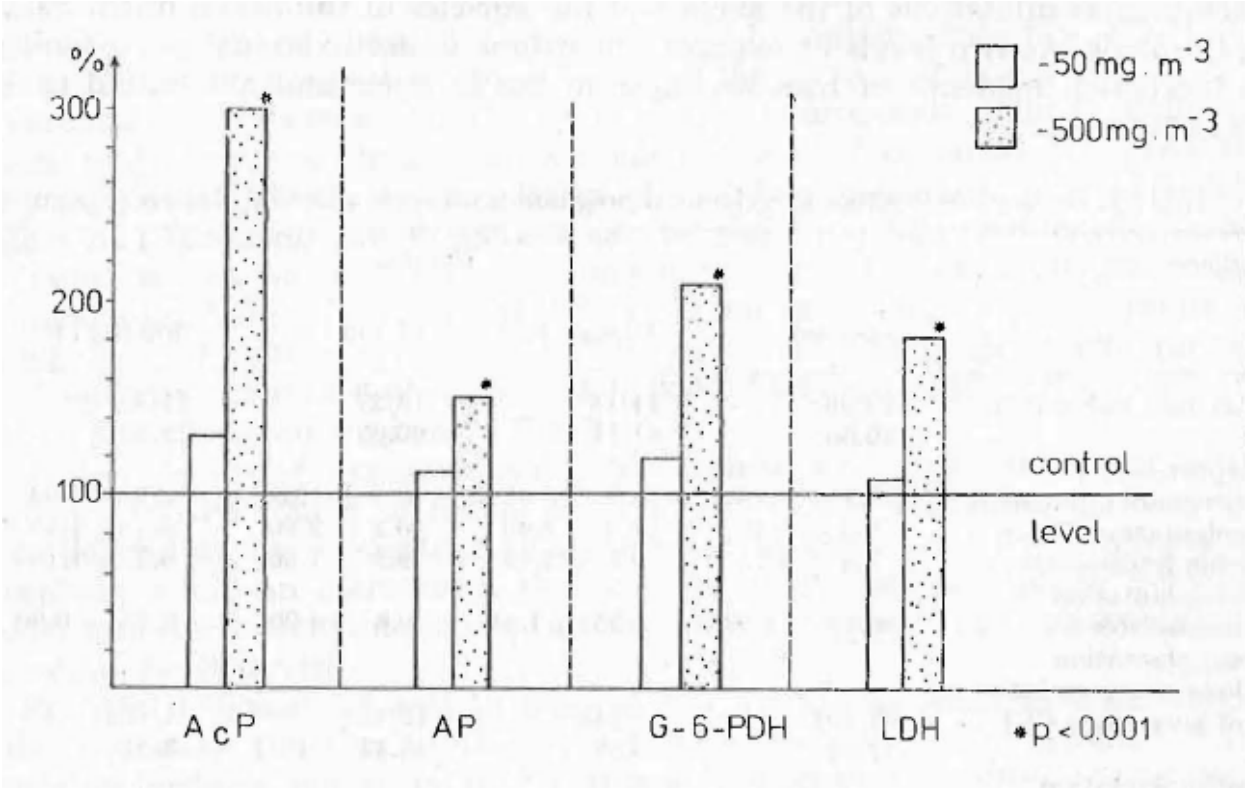
Endpoint	Control	2.31 ppm	11.53 ppm	115 ppm
Gestation index (%)	29/36 (80.56%)	11/18 (61.11%)	18/27 (66.67%)	11/15 (73.33%)
Mean corpora lutea/litter	10.1 ± 1.46	10.5 ± 2.53	10.8 ± 1.59	9.5 ± 1.03
Mean implantations/litter	9.0 ± 2.14	10.2 ± 2.80	10.2 ± 2.80	9.1 ± 1.48
Mean viable fetuses/litter	8.8 ± 1.75	9.5 ± 2.74	9.2 ± 1.90	8.7 ± 1.10
Resorptions/implantations (%)	15/271 (5.54%)	8/143 (5.59%)	15/205 (7.32%)	9/101 (8.91%)
Post-implantation losses/implantations (%)	15/271 (5.54%)	9/143 (6.29%)	22/205 (10.73)*	15/101 (14.85)*
Autolysis/implantations (%)	0/271 (0%)	1/143 (0.7%)	7/205 (3.41%)**	6/101 (15.94%)**
Mean fetal weight per litter (g)	3.64 ± 0.26	3.47 ± 0.31	3.20 ± 0.28**	3.17 ± 0.24*
% Fetuses with hemorrhages	80/251 (31.01%)	52.134 (38.81%)	80/175 (45.71%)**	51/96 (53.13)**

*p <0.05; **p <0.01

Results for the pups followed postnatally were not tabulated in the study, but were described in the text as follows:

- Pup weights were significantly decreased on PND 7 and 21 by 15% and 12.2%, respectively, in the 115 ppm group
- Pup weights were significantly decreased on PND 7 and 21 by 13% and 15%, respectively, in the 11.53 ppm group
- No effect of xylene treatment on pup mortality was observed
- "Metabolic disturbances" occurred in the liver, brain, myocardium, and lungs at xylene concentrations of 11.53 and 500 ppm (Figure XX, reproduced below, provided data for lung)

Figure B.6. Enzyme Activity from Lung Tissues of PND 21 Pups Prenatally Exposed to 11.53 or 115 ppm Xylene



AcP - acid phosphatase
AP - alkaline phosphatase
G-6-PDH - glucose-6-phosphate dehydrogenase
LDH - lactate dehydrogenase

The authors concluded that prenatal exposure to xylene at concentrations of 11.53 and 115 ppm resulted in embryotoxic and teratogen effects, and interfered with postnatal growth and functional development.

Nylen et al., 1989. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene

In a study of the testicular and germ-cell line toxicity of n-hexane, some experiments involved simultaneous administration of n-hexane with other solvents, including xylene. Male Sprague-Dawley rats were exposed over 61 days to 1000 ppm n-hexane by inhalation, either alone or in combination with the same concentration of toluene or xylene. Control data were collected on a xylene-only group, as well as untreated controls. Animals in the xylene group were exposed 18 hours per day, seven days per week, for 61 days. Test animals were evaluated for testicular and germ cell line at two weeks, 10 months, and 14 months after cessation of the 61-day exposure period.

Endpoints evaluated included:

- Gross and histological pathology of testis and epididymis
- Indirect immunofluorescence for nerve growth factor (NGF)
- Androgen biosynthetic capacity of testis
- Blood testosterone concentration
- Vas deferens morphology
- Noradrenaline (NA) concentration
- Epididymal sperm morphology
- Fertility

Three xylene-exposed rats were randomly selected for fertility studies at 13 months following the end of exposure. Each male was housed with three normal females for up to 35 days. At the end of the mating period, males with no pregnant females would have been scored as non-fertile. All three of the xylene-exposed males were fertile.

No gross pathological effects were observed in the testes of xylene-exposed animals. No changes in immunoreactivity to NGF-antibody were observed in the testes of xylene-exposed animals. Data for other endpoints of male reproductive toxicity are presented in Tables B.22 and B.23, below. The data provided no evidence for an adverse effect of xylene, under the experimental conditions tested, on the male reproductive system.

Table B.22. Endpoints of male reproductive toxicity in rats exposed to xylene for 61 days

Endpoint and time of assessment	Controls	1000 ppm xylene
% Intact spermatozoa (2 weeks post exposure)	97 (71-99)	99 (85-100)
% Intact spermatozoa (10 months post exposure)	98 (97-100)	97 (89-100)
% Normal sperm morphology (2 weeks post exposure)	100 (0-298)	99 (97-100)
% Normal sperm morphology (10 months post exposure)	99 (95-100)	97 (88-100)
Testis weight, g (2 weeks post exposure)	2.10 (2.00-2.19)	2.11 (2.06-2.14)
Testis weight, g (10 months post exposure)	2.43 (2.23-2.59)	2.37 (1.13-2.88)
Ventral prostate weight, mg (2 weeks post exposure)	556 (405-692)	480 (386-664)
NA-content, vas deferens, µg/g (2 weeks post exposure)	12.4 (9.1-14.9)	11.8 (7.5-14.7)

All values mean and range; N = 6 for all groups

Table B.23. Testosterone concentration in plasma and testicular in vitro metabolism of 14C progesterone at 2 weeks post 61 days of xylene exposure

Group	Testosterone nmol/l	14C- progesterone metabolites formed, nmol/mg protein/hr		
		17-hydroxy progesterone	Androstenedione	Testosterone
Controls	9.7 ± 5.8	0.070 ± 0.007	2.06 ± 0.71	0.061 ± 0.019
1000 ppm xylene	15.7 ± 10.6	0.094 ± 0.004	1.91 ± 0.27	0.062 ± 0.010

All values mean ± SD; N = 6 for all groups

It is interesting to note that concurrent exposure to 1000 ppm xylene apparently had protective effects on animals exposed to n-hexane. On its own, n-hexane caused severe testicular atrophy involving seminiferous tubules with loss of the NGF immunoreactive germ cell line. When co-administered with either 1000 ppm xylene or toluene, these effects were not observed.

Rosen et al., 1986. Postnatal evaluation of prenatal exposure to p-xylene in the rat

Rosen et al. (1986) exposed groups of 25 pregnant Sprague-Dawley rats to 0, 3500, or 7000 mg/m^3 (0, 807.31, or 1614.62 ppm) p-xylene for six hours/day on GD 7-16. Dams were weighed on GD 7 and 17, and allowed to give birth normally. Any dams not giving birth were sacrificed and their uteri examined for the presence of implantation sites.

Pups were counted and weight both at birth and on PND 3. On PND 4, litters were culled to eight pups, four males and four females (±1). At weaning on PND 21, littermates were housed together in groups of four same-sex pups per cage. Pup body weights were recorded weekly until weaning, then every other week post-weaning.

Ten litters from each group were used for behavioral testing. The acoustic startle response test was performed on single offspring on PND 13, 17, 21, and 63. The same animals were tested for locomotor activity in figure-eight mazes on PND 22 and 65.

A significant decrease was reported for maternal weight gain during treatment in the high-concentration group ($p < 0.05$). Exposure to p-xylene had no effect on the number of pregnant animals in each group.

Neither litter size nor pup weights showed an effect of treatment at birth or on PND 3. Treatment with p-xylene did not affect pup growth rate. Normal, significant ($p < 0.001$) changes in pup body weight occurred as a function of age and sex, with a significant age-sex interaction.

Locomotor activity in the figure-eight maze was not affected by treatment. Again, there were significant changes related to pup age and sex ($p < 0.001$), as well as a significant age-sex interaction ($p < 0.01$).

Xylene treatment did not affect any measure of the acoustic startle response. Age, sex, age-sex interactions, background noise, and age-background interactions were all significant factors at the $p < 0.001$ level.

The authors concluded that p-xylene, at concentrations associated with a significant decrease in maternal gestational weight gain, did not provide evidence of embryo or fetal toxicity, or adverse effects on postnatal CNS development.

Saillenfait et al., 2003. Developmental toxicity of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure

> Groups of 23-26 female Sprague-Dawley rats were exposed to technical xylene (15.3% ethylbenzene, 21.3% o-xylene,43.9% m-xylene, 19.4% p-xylene) or one of its individual isomers by inhalation. Each agent was provided at concentrations of 0, 100, 500, 1000, or 2000 ppm. The duration of exposure was six hours per day, on each of GD 6-20. Dams were sacrificed on GD 21, and their uteri removed for evaluation of the contents.

Each of the agents tested resulted in reduced gestational weight gain at 1,000 and 2,000 ppm. Decreases in corrected maternal weight gain (accounting for the pregnant uterus) and feed consumption were observed with 2000 ppm technical xylene, as well as with both 1000 and 2000 ppm of the other test compounds.

Table B.24. Maternal Gestational Weight Gain

Compound and concentration (ppm)		Number dams	Corrected gestational weight gain (g)
m-xylene	0	21	28 ± 9
	100	24	29 ± 13
	500	22	29 ± 10
	1000	22	19 ± 12*
	2000	24	5 ± 8**
o-xylene	0	21	28 ± 12
	100	22	29 ± 10
	500	24	26 ± 9
	1000	20	16 ± 12**
	2000	21	1 ± 12**
p-xylene	0	25	35 ± 12
	100	26	41 ± 11
	500	25	32 ± 13
	1000	25	23 ± 13**
	2000	22	7 ± 15**
Technical xylene	0	24	30 ± 12
	100	21	31 ± 20
	500	24	29 ± 13
	1000	20	20 ± 10
	2000	21	1 ± 12**

Values expressed as means ± S.D.
*p <0.05, **p <0.01

None of the test compounds, at any concentration, showed significant effects on maternal mortality, pregnancy rate, corpora lutea, implantation sites, percentages of live and dead fetuses per litter, resorption frequency, or fetal sex ratio. Fetal weights were significantly decreased with exposure to xylene or any of its three isomers at concentrations of 1,000 or 2,000 ppm (p <0.01 in each case). Significant decreases in fetal weight were also seen at 500 ppm o-xylene and technical xylene (p <0.05 and p <0.01, respectively).

Live fetuses were examined for external morphological abnormalities. Half of each litter was preserved and examined for internal anomalies by standard techniques. Remaining fetuses were fixed cleared and stained with Alizarin Red S for examination of skeletal elements.

None of the test agents, at any concentration, resulted in statistically significant changes in the frequencies of external or internal abnormalities. The frequency of skeletal variations, either among total fetuses or considered as a percent of litters having fetuses with skeletal variations, was not affected by technical xylene at any test concentration. Skeletal variations were increased in frequency among fetuses exposed to 2000 ppm of

any of the individual xylene isomers (see Table B.25 below). With exposure to o-xylene at concentrations of 1000 or 2000 ppm, significant increases in the number of fetuses with any variations (external, visceral, and skeletal combined) were seen (p <0.05 at both concentrations). Such an increase was also seen for p-xylene at 2,000 ppm (p <0.01), but not with m-xylene or technical xylene.

Table B.25. Effects of Xylene and Its Isomers on Fetal Parameters

Compound and concentration (ppm)		Mean fetal body weight per litter (g)	Number (%) fetuses with skeletal variations; number (%) litters with skeletal variations	Number (%) fetuses with any variations; number (%) litters with any variations
m-xylene	0	5.75 ± 0.35	23 (16.1); 13 (61.9)	32 (11.2); 16 (76.2)
	100	5.75 ± 0.36	27 (16.1); 17 (70.8)	34 (10.2); 21 (87.5)
	500	5.81 ± 0.38	23 (16.9); 13 (59.1)	28 (10.3); 14 (63.6)
	1000	5.41 ± 0.28**	35 (23.3); 17 (77.3)	43 (14.3); 17 (77.3)
	2000	4.91 ± 0.42**	44 (27.9)*; 20 (83.3)	50 (15.8); 20 (83.3)
o-xylene	0	5.76 ± 0.31	26 (18.1); 14 (66.7)	30 (10.4); 15 (71.4)
	100	5.71 ± 0.27	23 (16.2); 13 (61.9)	29 (10.2); 16 (76.2)
	500	5.49 ± 0.33*	40 (23.7); 18 (75.0)	44 (13.0); 19 (79.2)
	1000	5.30 ± 0.31**	37 (27.8); 17 (85.0)	45 (16.9)*; 18 (90.0)
	2000	4.64 ± 0.24**	47 (35.1)**; 18 (94.7)	47 (17.5)*; 18 (94.7)
p-xylene	0	5.73 ± 0.25	30 (17.3); 14 (56.0)	36 (10.4); 17 (68.0)
	100	5.69 ± 0.30	34 (18.5); 18 (69.2)	39 (10.6); 19 (73.1)
	500	5.64 ± 0.25	40 (22.6); 20 (80.0)	40 (11.3); 20 (80.0)
	1000	5.41 ± 0.30**	42 (25.3); 16 (66.7)	46 (13.8); 16 (66.7)
	2000	4.79 ± 0.55**	54 (40.0)**; 18 (81.8)	61 (22.6)**; 19 (86.4)
Technical xylene	0	5.83 ± 0.29	30 (18.9); 16 (66.7)	38 (12.0); 17 (70.8)
	100	5.73 ± 0.25	25 (18.9); 13 (65.0)	28 (10.6); 14 (70.0)
	500	5.60 ± 0.27**	31 (19.0); 17 (70.8)	36 (11.0); 17 (70.8)
	1000	5.41 ± 0.25**	38 (27.7); 15 (75.0)	47 (17.2); 16 (80.0)
	2000	4.89 ± 0.26**	38 (25.5); 16 (76.2)	45 (15.1); 17 (81.0)

N = 19-26; values expressed as means ± S.D.
*p <0.05, **p <0.01

Technical xylene and each of its isomers produced developmental toxicity in the form of reduced fetal weights at the two highest concentrations of 1000 and 2000 ppm. These concentrations were also associated with decreases in maternal gestational weight gain, but not with maternal mortality. This level of maternal toxicity would be considered "minimal" under U.S. EPA guidelines for risk assessment for developmental toxicity (U.S. EPA, 1991). Decreased fetal weights were also seen with 500 ppm technical xylene and m-xylene, concentrations that were not associated with significant maternal effects.

Significant changes in the frequencies of skeletal variations and/or total variations were seen at 1000 and/or 2000 ppm with some, but not all, forms of xylene tested. Nonetheless, there does not appear to be evidence for strong, qualitative differences among xylene isomers.

Ungvary et al., 1980. Studies on the embryotoxic effects of ortho-, meta-, and para-xylene

Pregnant CFY rats were exposed to individual xylene isomers by inhalation at concentrations of 0, 150, 1500, or 3000 mg/m^3 (0, 35, 346, or 692 ppm). Animals were kept in the exposure chambers for 24 hours per day on GD 7-14. Additional pregnant rats were exposed to o-xylene at the same concentrations, but restricted to two hours only on GD 18.

Death occurred in 4/30 females exposed to the highest concentration of m-xylene, but no maternal mortality was seen in any of the other groups. The numbers of non-pregnant animals at term varied among groups and did not seem to show a dose-related pattern (see Tables B.26, B.27, and B.28 below). Seven out of 20 litters in the high concentration group of p-xylene were totally resorbed, as were 2/20 litters in the high concentration group of o-xylene.

Gestational weight gain decreased with increasing dose for each xylene isomer, but only reached statistical significance at the high concentration of m-xylene ($p < 0.05$). Maternal food consumption was decreased during the treatment period with exposure to the high concentration of o-xylene, and the medium and high concentrations of m- and p-xylene. Feed consumption returned to normal, or exceeded control levels following the end of the treatment period. The liver/body weight ratio was significantly lower than controls ($p < 0.05$) for all concentrations of o-xylene, but did not show a clear dose effect. Liver/body weight ratio was not affected in any of the other groups.

A significant decrease in the mean number of implantation sites per dam was seen only at the high concentration of m-xylene ($p < 0.05$). Mean litter size and fetal loss expressed as a percentage of implantation sites were altered only at the highest concentration of p-xylene.

Fetal endpoints for each isomer were as follows:

- o-xylene
  - Mean fetal weight was significantly decreased at 346 and 692 ppm ($p < 0.001$, at both concentrations)
  - Mean placental weight was significantly decreased at 35 ppm ($p < 0.05$)
  - Mean placental weights were significantly increased at 346 ppm ($p < 0.05$), and at 692 ppm ($p < 0.001$)
  - The number of fetuses showing skeletal retardation out of the total number of fetuses was significantly increased at 692 ppm ($p < 0.05$)
  - No other significant external, internal or skeletal anomalies or malformations
- m-xylene
  - Mean fetal weight was significantly decreased at 692 ppm ($p < 0.01$)
  - No effect on placental weight
  - Increased frequency of extra ribs at 692 ppm ($p < 0.05$)
  - No other significant external, internal or skeletal anomalies or malformations
- p-xylene
  - Mean fetal weight significantly decreased at 692 ppm ($p < 0.001$)
  - Mean placental weights significantly decreased at 35 ppm ($p < 0.01$), and at 346 and 692 ppm ($p < 0.001$, at both concentrations)
  - The frequency of skeletal retardation was significantly increased at all three concentrations of p-xylene ($p < 0.05$, at all concentrations)
  - The frequency of extra ribs was significantly increased at 692 ppm ($p < 0.01$)
  - No other significant external, internal or skeletal anomalies or malformations

The additional groups of three rats each, which were exposed to o-xylene at 0, 35, 346 or 692 ppm, for two hours on GD 18, were used for studies of xylene in blood and amniotic fluid. The presence of o-xylene in maternal blood was found to be proportional to the test concentration. O-xylene was demonstrated to have crossed the placenta, as it was found both in fetal blood and in amniotic fluid.

Table B.26. Effects on Pregnant CFY Rats Exposed to o-Xylene

Endpoint	Air control	35 ppm	346 ppm	692 ppm
N live litters/N treated ♀	13/15	17/20	18/20	17/20
Mean litter size	12.9 ± 0.9	12.8 ± 0.8	13.4 ± 0.6	13.8 ± 0.6
Mean fetal weight (g)	4.07 ± 0.03	4.02 ± 0.02	3.72 ± 0.02**	3.75 ± 0.03**
Mean placental weight (g)	0.63 ± 0.007	0.61 ± 0.008*	0.65 ± 0.007*	0.68 ± 0.007**
Number fetuses showing skeletal retardation/number alizarin-stained fetuses	13/84	27/110	30/123	48/121*

Continuous variables represented as mean ± S.E.
*p <0.05; **p <0.001

Table B.27. Effects on Pregnant CFY Rats Exposed to m-Xylene

Endpoint	Air control	35 ppm	346 ppm	692 ppm
N live litters/N treated ♀	23/25	15/20	19/20	18/30
Maternal weight gain, % initial weight	65.8 ± 1.60	67.3 ± 2.63	64.7 ± 2.42	48.3 ± 2.64*
Mean implantations/dam	13.52 ± 0.37	14.27 ± 0.48	13.89 ± 0.66	11.44 ± 1.04*
Mean litter size	12.4 ± 0.4	13.2 ± 0.6	13.0 ± 0.8	10.9 ± 1.0
Mean fetal weight (g)	3.97 ± 0.02	3.85 ± 0.03	3.94 ± 0.03	3.60 ± 0.03*
Number fetuses with extra ribs/number alizarin-stained fetuses	2/142	1/102	3/123	8/97*

Continuous variables represented as mean ± S.E.
*p <0.05

Table B.28. Effects on Pregnant CFY Rats Exposed to p-Xylene

Endpoint	Air control	35 ppm	346 ppm	692 ppm
N live litters/N treated ♀	18/20	12/20	16/20	6/20
Mean litter size	12.6 ± 0.8	13.1 ± 1.0	12.3 ± 0.8	8.5 ± 2.45***
Mean fetal weight (g)	4.05 ± 0.03	4.02 ± 0.03	3.97 ± 0.02	3.56 ± 0.05***
Mean placental weight (g)	0.73 ± 0.007	0.64 ± 0.009**	0.66 ± 0.009***	0.61 ± 0.013***
Number fetuses showing skeletal retardation/number alizarin-stained fetuses	16/116	24/77*	38/100*	15/26*
Number fetuses with extra ribs/number alizarin-stained fetuses	6/116	1/77	9/100	10/26**

Continuous variables represented as mean ± S.E.
*p <0.05; **p <0.01; ***is p <0.001

The highest concentration of each of the three xylene isomers was found to exert some degree of maternal toxicity. All three isomers also were associated with retarded fetal development expressed as decreased fetal weight. Treatment with o-xylene and p-xylene was associated with symptoms of skeletal retardation. Severity of these effects was concentration-dependent, but also depended upon the particular isomer. In decreasing order of potency, their effectiveness was p-, o-, and m-xylene. Increased incidences of extra ribs were seen with m- and p-xylene, but not with o-xylene. The authors proposed that differences among the isomers in metabolism, and/or maternal toxicity, could explain these differences.

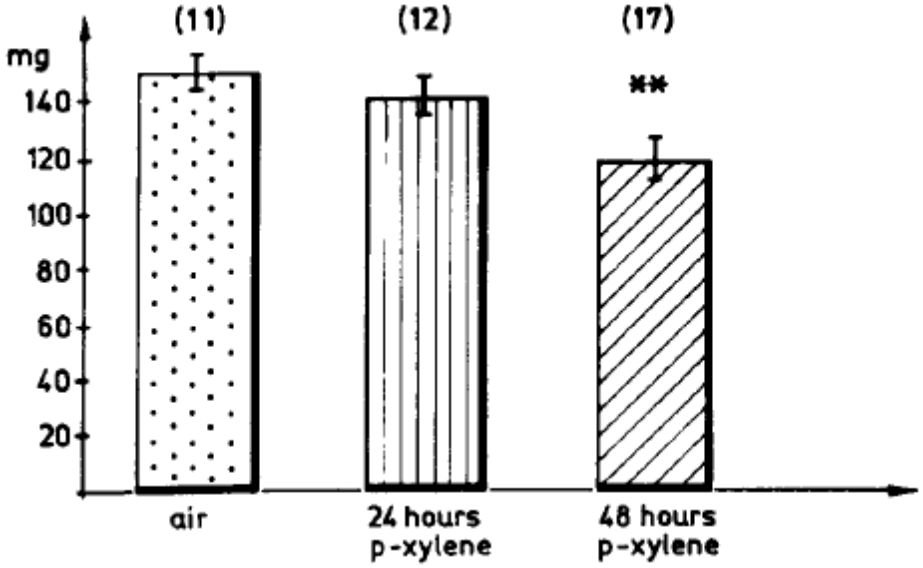
Ungvary et al., 1981. Study on the role of maternal sex steroid production and metabolism in the embryotoxicity of para-xylene

Ungvary et al. (1981) exposed CFY rats to 0 or 3000 mg/m^3 (0, 692 ppm) p-xylene on GD10 or GD 9-10. Exposure was continuous either for 24 hours, or for 48 hours. There were 8 to 14 pregnant females in each group. Uterine and ovarian venous blood flow, ovarian progesterone and 17ß-estradiol secretion, and levels of progesterone and 17ß-estradiol in peripheral blood were measured on GD 11. Also at this time, the uteri were removed and weighed, the number of embryos counted, and their weight calculated (as total uterine weight/number of embryos).

No significant effects were seen on blood flow in the ovary or uterus with exposure to p-xylene. Nor were there any significant effects of p-xylene on ovarian progesterone or 17ß-estradiol secretion. Peripheral blood levels of progesterone and 17ß-estradiol secretion were not affected by 24 hours of exposure to p-xylene, but both hormones were significantly decreased (p<0.05) by 48 hours of exposure.

Uterine weight divided by the number of embryos found in each uterus was significantly decreased (p<0.01) following 48 hours exposure to 692 ppm p-xylene. Data on embryo counts are not provided, but the text states that "there was no marked lethality." Therefore, the effect on weight was apparently due to smaller embryos, rather than fewer embryos.

Figure B.7. Weight of Uterine Contents on GD 11 (Uterine Weight/Number Embryos)



Bars are mean ± S.E.; **p<0.01, (N)

The authors concluded that since their data gave no evidence for altered uterine circulation, p-xylene did not affect embryo growth through this mechanism. In their view, the effect of 48 hours exposure to 692 ppm p-xylene on peripheral levels of progesterone and 17ß-estradiol should be considered as a factor contributing to the embryotoxic effects of p-xylene.

Ungvary and Tatrai, 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits

Ungvary and Tatrai (1985) studied the effects of technical xylene and/or its individual isomers by inhalation in pregnant rats, mice, and rabbits. Exposure protocols were as follows:

- CFY rats
    - Technical xylene at concentrations of 0, 250, 1900 or 3400 mg/m^3 (0, 58, 438, or 784 ppm)
    - 24 hrs/day on GD 7-15; N = 20-23

- CFLP mice
  - Technical xylene at concentrations of 0, 500 or 1000 mg/m^3 (0, 115 or 231 ppm)
  - Individual xylene isomers at concentrations of 0 or 500 mg/m^3 (0 or 115 ppm)
  - 24 hrs/day on GD 6-15; N = 15-18 (115 controls)
- NZ rabbits
  - Technical xylene or one of its isomers at concentrations of 0, 500 or 1000 mg/m^3 (0, 115 or 231 ppm)
  - 24 hrs/day on gestation days 7-20; N = 9-10 (60 controls).

Results for each species were as follows:

- Rats
  - 1/20 high-concentration group dams died
  - Increased percentage of dead or resorbed fetuses at the high concentration of 784 ppm ($p < 0.05$)
  - Fetal effects at 58 ppm
    - Increased percentage of fetuses with skeletal retardation ($p <0.01$)
  - Fetal effects at 438 ppm
    - increased percentage of fetuses with skeletal retardation ($p <0.01$)
  - Fetal effects at 784 ppm
    - Increased percentages of weight retarded fetuses ($p <0.05$)
    - Increased percentages of fetuses with skeletal retardation ($p <0.01$)
    - Increased Percentages of fetuses with minor anomalies, such as extra ribs ($p <0.05$)
- Mice
  - No effect of xylene or any of its isomers on maternal mortality
  - No effect of xylene or any of its isomers on the numbers of live fetuses, or the percentage of dead or resorbed fetuses
  - No effects of xylene or any of its isomers on the percentages of minor anomalies, such as extra ribs, or total malformations
  - Increased frequencies of weight retardation and skeletal retardation for all three isomers ($p <0.05$ as compared to controls)
  - Technical xylene
    - At 115 ppm, no fetal effects
    - At 231 ppm, significant increases in the percentages of weight retarded fetuses, and fetuses with skeletal retardation ($p <0.05$ for both endpoints)
- Rabbits
  - No effect of xylene or any of its isomers on maternal mortality or percent gestational weight gain
  - Significant increase in percent relative maternal liver weight for the high concentration of technical xylene only ($p < 0.05$)
  - 1/8 and 3/10 dams died at 231 ppm p-xylene and technical xylene, respectively

- 3/8 and 6/10 litters were aborted at 231 ppm p-xylene and technical xylene, respectively
- 1/10, 4/8, and 1/10 litters were totally resorbed or dead at 115 ppm p-xylene, 231 ppm p-xylene, and 231 ppm technical xylene, respectively
- The percentage of dead or resorbed fetuses, among total fetuses, was significantly increased at 115 ppm m-xylene ($p < 0.05$)
- None of the 3 xylene isomers (o-, m-, or p-) affected mean fetal weight, or the frequencies of skeletal retardation or the presence of anomalies
- Technical xylene at 115 ppm was associated with a significant decrease in the mean weight of female fetuses ($p <0.05$)

Table B.29. Rats Exposed to Xylene 24 Hours per Day on GD 7-15

Endpoint	Air control	58 ppm	438 ppm	784 ppm
Number dams	20	23	22	19 (1 death)
Number live fetuses	227	296	274	205
Dead or resorbed fetuses	5%	8%	7%	13%*
Weight retarded fetuses	2%	2%	3%	13%*
Skeletal retarded fetuses	13%	32%**	33%**	31%**
Minor anomalies (extra ribs)	0	1%	0	9%*
All malformations	1%	1%	1%	1%

For each group, N = 20-23; data expressed as % affected of total fetuses
*p <0.05; **p <0.01

Table B.30. Mice Exposed to Xylene 24 Hours per Day, on GD 6-15

Endpoint	Air control	o-xylene 115 ppm	m-xylene 115 ppm	p-xylene 115 ppm	Technical xylene 115 ppm	Technical xylene 231 ppm
Number dams	115	17	18	17	15	15
Number live fetuses	1170	172	185	165	159	156
Dead or resorbed fetuses	6%	7%	7%	10%	7%	7%
Weight retarded fetuses	7%	28%*	27%*	29%*	8%	30%*
Skeletal retarded fetuses	5%	11%*	11%*	12%*	6%	13%*
Minor anomalies (extra ribs)	4%	4%	4%	4%	4%	4%
All malformations	4%	3%	4%	4%	6%	6%

Data expressed as % affected of total fetuses
*p <0.05

Table B.31. Rabbits Exposed to Xylene 24 Hours per Day on GD 7-20

Endpoint	Air control	o-xylene		m-xylene		p-xylene		Technical xylene	
		115 ppm	231 ppm	115 ppm	231 ppm	115 ppm	231 ppm	115 ppm	231 ppm
Dams died/total dams	0/60	0/9	-	0/9	-	0/10	1/8	0/10	3/10
Number aborted litters/surviving dams	0/60	0/9	-	0/9	-	0/10	3/7	0/10	6/7
Number (totally) resorbed litters/non-aborted litters	0/60	0/9	-	0/9	-	1/10	4/4	0/10	1/1
Maternal weight gain (% initial weight)	12.7% ± 1.08	8.7% ± 1.43	-	9.2% ± 1.77	-	11.7% ± 3.2	-	12.1% ± 3.3	8.3% ± 3.4

Relative liver weight (% body weight)	3.0% ± 0.12	3.2% ± 0.14	-	3.2% ± 0.12	-	3.2% ± 0.16	-	3.1% ± 0.20	3.6%* ± 0.22
Number live fetuses	501	72	-	59	-	73	-	70	0
Dead or resorbed fetuses (% total fetuses)	5.2%	3.0%	-	12.8%*	-	8.9%	-	5.1%	-
Mean fetal weight ♂ (g)	33.0 ± 1.06	34.0 ± 1.75	-	33.1 ± 1.88	-	33.5 ± 1.66	-	32.4 ± 1.75	-
Mean fetal weight ♀ (g)	32.7 ± 0.86	33.9 ± 1.82	-	33.2 ± 1.90	-	33.3 ± 1.72	-	29.4* ± 1.13	-
% Skeletal retardation	40%	42%	-	41%	-	43%	-	48%	-
% Minor anomalies	34%	35%	-	33%	-	37%	-	34%	-
% Skeletal malformations	2%	-	-	-	-	1%	-	3%	-
% Internal malformations	5%	3%	-	4%	-	3%	-	1%	-
% External malformations	2%	-	-	-	-	1%	-	6%	-
% All malformations	6%	3%	-	4%	-	4%	-	6%	-

Fetal data expressed as mean % affected of total fetuses, not on a per litter basis.
Paper does not clearly state whether error is expressed as S.D. or SE.
Despite inclusion in the table, no data were presented for o- or m-xylene at the higher concentration of 231 ppm; no explanation was provided.
*p<0.05

Washington et al., 1983. Induction of morphologically abnormal sperm in rats exposed to o-xylene

Sprague Dawley rats, 10-16 weeks old, were given 0.5 or 1.5 ml/kg-bw o-xylene in corn oil, by i.p. injection. Controls were given the corn oil vehicle by injection. The number of animals in each group is not made clear in the study report. Treatments were given over a two-day period.

Sperm morphology was evaluated at five weeks following treatment. Preparations of epididymal sperm were evaluated by light microscopy. From each group, a total of 500-1000 sperm per animal were examined. Observed sperm abnormalities were described as:

- Amorphous head
- Head lacking typical hook
- Banana-like head
- Abnormal tail folding

In combination with xylene exposure, ambient temperature was a significant variable affecting sperm abnormality. Treated animals kept at 20-24°C showed no significant increase in the frequency of abnormal sperm over levels seen among control animals. When housed at temperatures between 24 and 30°C, rats given 0.5 ml/kg o-xylene showed a significant increase in abnormal sperm of 1.23% over controls ($p < 0.05$). Untreated controls showed no effect of temperature.

Yamada, 1993. Influence of lacquer thinner and some organic solvents on reproductive and accessory reproductive organs in the male rat

Male Wistar rats, seven to eight weeks of age, were exposed to xylene in an inhalation apparatus, twice per day for seven days. Xylene concentration was not measured directly, but animals were left in the apparatus until the righting reflex disappeared. Hence, the neurological effect indicates that the animals were exposed to a biologically significant concentration. Animals were sacrificed on the eighth day for evaluation:

- Weights of testes and accessory male reproductive organs
- Plasma testosterone levels
- Prostate acid phosphatase activity
- Epididymal sperm counts

Animals were weighed daily during the treatment period; xylene-treated animals showed a significant difference from controls only on the last day of treatment ($P <0.05$).

Under the conditions of this study, xylene exposure was associated with reduced weights of testis and accessory male reproductive organs (Table B.32). Lowered plasma testosterone levels were also observed, as well as a reduction in prostate acid phosphatase activity. There was also a reduction in the numbers of epididymal sperm.

Table B.32. Effects of Xylene Inhalation on Male Reproductive Organs

Endpoint	Control	xylene-exposed
Testes weight (g)	2.54 ± 0.068	2.51 ± 0.164
Epididymis weight (mg)	412 ± 6.6	369 ± 13.0*
Vas deferens weight (mg)	111 ± 4.3	90 ± 2.0**
Seminal vesicles weight (mg)	172 ± 1.3	108 ± 5.1**
Prostate weight (mg)	171 ± 9.8	112 ± 4.6**
Plasma testosterone level (ng/ml)	2.19 ± 0.324	0.73 ± 2.03**
Epididymal sperm X 10^6	2.54 ± 0.301	1.08 ± 0.529*
Acid phosphatase activity in prostate (KA unit/mg protein)	0.21 ± 0.032	0.14 ± 0.018*

All values mean ± S.E.; N = 5 for all groups
By Student' s *t*-test: *P <0.05; **P <0.01