Xylenes Tier I VCCEP Submission

Appendix B

Xylenes VCCEP Robust Summaries

October 6, 2005

Xylenes VCCEP Robust Summaries

Tier 1

Tier 1 Acute Toxicity – Inhalation

Tier 1: Acute Inhalation Robust Summary 1- Hine and Zuidema 1970

Test Substance	Mixed xylenes (C8 aromatics): ortho-, meta-, para-xylenes and ethyl benzene [percentages not reported]. Boiling range 138-141^{0}C [281-286^{0}F], 98% pure. From Shell Chemical Co., Petrochemical Division.
Method	Not specified. Standard procedure
Method/guideline followed	
Type (test type)	Inhalation LC_{50}
GLP	No
Year	1970
Species/Strain	Rats, Long Evans
Sex	Male
No. of animals per sex per dose	10
Vehicle	None
Route of administration	Inhalation, single 4hr exposure
Test Conditions	Ten male rats (150-300g)/group were exposed to mixed xylenes in a 250 liter chamber for 4 hours. Saturated vapor was generated by bubbling air through the mixed xylene sample, heated to 77^{0}F [25^{0}C] in a water bath. Vapor concentrations in the chamber were monitored by Wilkens Aerograph GLC and compared with vapor concentrations obtained from a bottle known to contain vapors saturated at 25^{0}C. For graded concentrations less than saturated, air entering the chamber was divided so that only part of it passed through the xylene sample. Rats were observed for 14 days after dosing for mortality and clinical signs of toxicity. The LC_{50} was determined by the method of Litchfield and Wilcoxon.

Results	LC_{50} = 6350ppm [C.L. 4670-8640ppm]. Approx. 50-90% of saturation at 25^0C
Remarks	All deaths occurred during exposure. Survivors were comatose but recovered shortly after removal from the chamber.
Conclusions (study author)	Mixed xylenes with an LC_{50} = 6350ppm was considered slightly toxic to rats exposed by inhalation for 4 hrs.
Data Quality Reliability	**2**. Reliable with restrictions. Percentages of xylene isomers in the mixed xylene sample were not reported. This publication presented summarized results of tests with 4 aromatic and 6 aromatic-free solvents and did not report actual doses and specific mortality data.
References	Hine, C.H., and Zuidema, H.H. 1970. The toxicological properties of hydrocarbon solvents. Indust Med 39: 39-44.
Other Last changed	2/14/03

Tier 1: Acute Inhalation Robust Summary 2- Carpenter et. al. 1975

Test Substance	Mixed xylenes: 65% meta-, 7.6% ortho- and 7.8% para-xylene, 19.3% ethyl benzene, 0.14% toluene, 0.04% C9+ aromatics, 0.04% non-aromatics, and no benzene. Composition determined by GC. Mol wt =106, Sp. Gr.= 0.87. Supplied by un-named American Producer
Method/guideline followed	Not specified. Standard procedure
Type (test type)	Inhalation LC_{50}
GLP	No
Year	1975
Species/Strain	Rats, Harlan Wistar
Sex	Male
No. of animals per sex per	15

dose	
Vehicle	None
Route of administration	Inhalation, single 4hr exposure
Test Conditions	Fifteen young male rats were exposed for 4 hrs. to mixed xylenes at measured concentrations: 2.5, 5.8, 12, 26, and 43mg/l [580, 1300, 2800, 6000, and 9900ppm]. Measured concentrations averaged 50-60% of metered concentrations, which reflected the total of all losses in the generation of vapor, plus losses by adsorption onto chamber walls, animal hair and body surfaces. Chamber concentrations were monitored and characterized by a gas chromatograph with flame ionization detector. In each group, 10 rats were observed for 14 days after exposure for signs of toxicity and mortality. LC_{50} with fiducial range, was calculated by the Thompson [1947] method of moving averages using the tables of Weil [1952] and other unpublished tables. Five rats/group were sacrificed for gross necropsy at the end of exposure.
Results	LC_{50} = 29 (22-37) mg/l = 6700 (5100-8500) ppm
Remarks	Mortality occurred in 10/10 rats exposed to 43mg/l and 4/10 rats exposed to 26mg/l; no rats died at 12, 5.8, or 2.5mg/l. Deaths in the 43mg/l group occurred within 2.25hr. In the 26mg/l group, rats were prostrate within 30min and all died within 3.5hrs; all survivors were prostrate as exposure ended but recovered promptly and appeared normal throughout 14 days of observation. Signs of exposure included eye irritation and irritation of the extremities. Rats exposed to 12mg/l became prostrate between 2 –3.5hr but recovered within 1 hr, although coordination remained poor, by 24hr post-exposure rats had fully recovered. No effects were seen in rats exposed to 2.5mg/l but 5.8mg/l induced poor coordination after 2hr exposure, which did not persist after exposure was terminated. The only significant histological findings among 43mg/l rats that died were 2 cases each of atelectasis, hemorrhage, and interlobular edema of the lungs.
Conclusions (study author)	LC_{50} = 29mg/l = 6700ppm. Non-lethal clinical signs included irritation of the eye and extremities, poor coordination, and prostration that subsided after cessation of exposure.

Data Quality Reliability	1. Reliable without restrictions.
References	Carpenter, C.P., Kinkead, E.R., Geary, Jr., D.L., Sullivan, L.J., and King, J.M. 1975. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapors of mixed xylenes. Toxicol Appl Pharmacol 33: 543-558.
Other Last changed	2/14/03

Tier 1: Acute Inhalation Robust Summary 3- Bonnet et. al. 1979

Test Substance	Meta-xylene (CAS #108-38-3; 97% pure); ortho-xylene (CAS #95-47-6; 98% pure); para-xylene (CAS #106-42-3, 98% pure) from Merck.
Type (test type)	Acute Inhalation Toxicity - LC_{50}
Method/guideline followed	Not specified, standard method
GLP	No
Year	1979 publication
Species/Strain	Mouse/ S.P.F., OF1
Sex	Female
No. of animals per sex per dose	20-25/group
Vehicle	None
Route of administration	Inhalation, 6hr exposure
Test Conditions	Female mice (21±1g) were exposed for 6 hr to each isomer of xylene, initially at concentrations separated by geometric log progression of 2; definitive studies were performed at log progressions of 1.05 or 1.1. [From fig. 1, estimated 6-8 total doses/ isomer to determine LD_{50}]. Twenty-eight exposure chambers (200L) were operated according to method of Gradiski et al. (1978). Temperature and humidity were maintained at 24±1^{0}C and 50±2%, respectively; chamber reduced pressure approx. 3mmH_20. Test liquid was vaporized and diluted with clean air to appropriate concentrations when entering the chamber. Chamber atmospheres were automatically sampled at 3min intervals,

	analyzed by gas chromatography and reported in ppm. Mice were not weighed in this study. Clinical signs during exposure were not reported. Mice were observed for 14 days post-exposure. LC_{50} with 95% confidence limits were determined by the method of Bliss (1938).
Results LC_{50} with confidence limits.	LC_{50} m-xylene = 5267ppm [5025-5490ppm] LC_{50} o-xylene = 4595ppm [4468-4744ppm] LC_{50} p-xylene = 3907ppm [3747-4015ppm]
Remarks	Some deaths occurred during exposure and at 5 and 10 days post-exposure with several hydrocarbons including para-xylene [specifics by chemical not reported].
Conclusions (study author)	Ranking of acute inhalation toxicity for xylene isomers: p-xylene< o-xylene < m-xylene over an exposure range of 3700-5500ppm.
Data Quality Reliability	2, Reliable with restrictions. Purity and source of test materials provided. LD_{50} calculations appropriate. Chamber atmospheres monitored but data not presented, no clinical signs observed, no gross necropsy reported.
References	Bonnet, P., Raoult, G., and Gradiski, D. 1979. Concentrations léthale$_{50}$ des principaux hydrocarbures aromatiques. Arch. Mal. Prof. 40: 805-810.

Tier 1: Acute Inhalation Robust Summary 4- Bonnet et. al. 1982

Test Substance	Meta-xylene (CAS #108-38-3; 97% pure); ortho-xylene (CAS #95-47-6; 98% pure); para-xylene (CAS #106-42-3, 98% pure) from Merck.
Type (test type)	Acute Inhalation Toxicity - LC_{50}
Method/guideline followed	Not specified, standard method
GLP	No
Year	1982 publication
Species/Strain	Rat/ Sprague Dawley, O.F.A., IFFA-CREDO
Sex	Male
No. of animals per sex per dose	12/group
Vehicle	None
Route of	Inhalation, 6hr exposure

administration	
Test Conditions	Male rats (130-160g) were exposed for 6 hr to each isomer of xylene, initially at concentrations separated by geometric log progression of 2; definitive studies were performed at log progressions of 1.05. [From fig. 1, estimated 8-10 total doses/ isomer to determine LD_{50}]. Exposure chambers (28 units) were operated according to method of Gradiski et al. (1978). Temperature and humidity were maintained at 24±1^{0}C and 50±2%, respectively. Test liquid was vaporized and diluted with clean air to appropriate concentrations when entering the chamber. Chamber atmospheres were monitored and analyzed by gas chromatography and reported automatically in ppm. Rats were weighed prior to exposure and at 7 and 14 days post-exposure [data not presented]. During exposure, rats were observed frequently for clinical signs that included hypotonia, stereotypy, somnolence, tremors and muscular weakness (shakes), and lacrimation. Rats were observed for 14 days post-exposure, necropsied, and principal organs examined grossly. LC_{50} with 95% confidence limits were determined by the method of Bliss (1938).
Results **LC_{50} with confidence limits.**	LC_{50} m-xylene = 5984ppm [5796-6181ppm] LC_{50} o-xylene = 4330ppm [4247-4432ppm] LC_{50} p-xylene = 4591ppm [4353-5049ppm]
Remarks	Clinical signs observed during exposure were hypotonia and somnolence in animals exposed to m- and o-xylene; hypotonia, stereotypy, somnolence, tremors and muscular weakness in animals exposed to p-xylene. Some deaths occurred during exposure and within 24 hr post-exposure with several hydrocarbons including para-xylene [specifics by chemical not reported].
Conclusions (study author)	Ranking of acute inhalation toxicity for xylene isomers: o-xylene< p-xylene < m-xylene over an exposure range of 4300-6000ppm.
Data Quality Reliability	2, Reliable with restrictions. Purity and source of test materials provided. Chamber atmospheres monitored but data not reported, clinical signs observed and reported, and LD_{50} calculations appropriate.
References	Bonnet, P., Morele, Y., Raoult, G., Zissu, D., and Gradiski, D. 1982. Détermination de la concentration léthale$_{50}$ des principaux hydrocarbures aromatiques chez le rat. Arch. Mal. Prof. 43:261-265.

Acute Toxicity – Oral

Tier 1: Acute Oral Robust Summary 1- Hine and Zuidema 1970

Test Substance	Mixed xylenes (C8 aromatics): ortho-, meta-, para-xylenes and ethyl benzene [percentages not reported]. Boiling range 138-141^{0}C [281-286^{0}F], 98% pure. From Shell Chemical Co., Petrochemical Division.
Method	
Method/guideline followed	Not specified. Standard procedure
Type (test type)	Oral LD_{50}
GLP	No
Year	1970
Species/Strain	Rats, Long Evans
Sex	Male
No. of animals per sex per dose	6
Vehicle	None
Route of administration	Oral, single dose
Test Conditions	Mixed xylenes were administered intragastrically to 6 rats (150-300g)/group, over a range of doses up to 25ml/kg. Rats were observed for 14 days after dosing for mortality and clinical signs of toxicity. The LD_{50} was determined by the method of Litchfield and Wilcoxon..
Results	LD_{50} = 10ml/kg [C.L. 7.5-13.3ml/kg] = 8.6mg/kg. Most deaths occurred during the first 72hrs after dosing. Animals became uncoordinated, prostrate or comatose at doses that produced partial mortality within the group and often at doses that were not lethal.
Conclusions (study author)	Mixed xylenes with an LD_{50} = 10ml/kg was considered practically non-toxic or relatively harmless in this test system.
Data Quality Reliability	**2**. Reliable with restrictions. Percentages of xylene isomers in the mixed xylene sample were not reported. This publication presented summarized results of tests with 4 aromatic and 6 aromatic-free solvents and did not report actual doses and specific mortality data.
References	Hine, C.H., and Zuidema, H.H. 1970. The toxicological properties of hydrocarbon solvents. Indust Med 39: 39-44.
Other Last changed	2/14/03

Tier 1: Acute Oral Robust Summary 2 - Smyth et. al. 1962

Test Substance	Meta-xylene, commercial (CAS #108-38-3)
Method/guideline followed	Not specified. Standard procedure employed
Type (test type)	LD_{50} [oral]
GLP	No
Year	1962 publication
Species/Strain	Rats/ Carworth-Wistar
Sex	Male
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral intubation
Test Conditions	Male rats (4-5 wks old, 90-120g), maintained on Rockland rat diet and water ad lib, were treated with undiluted m-xylene in single doses, arranged in logarithmic series differing by a factor of 2. Rats were observed for 14 days post-dosing. LD_{50} and its fiducal range were estimated by the method of Thompson [1947] using the tables of Weil [1952] with limits of ±1.96 standard deviations.
Results **LD_{50} with confidence limits.**	7.71ml/kg [6.24-9.53] = 6.66g/kg.
Remarks	Data appeared in a table of more than 300 compounds, no additional specific information provided.
Conclusions (study author)	LD_{50} = 7.71ml/kg [6.24-9.53] = 6.66g/kg.
Data Quality Reliability	2, Reliable with restrictions. No information was presented on m-xylene induced clinical signs, timing of deaths. Purity and source of m-xylene was not provided.
References	Smyth, H.F., Carpenter, C.P. Weil, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI. Amer Industr Hyg Assoc J 23: 95-107

Tier 1: Acute Oral Robust Summary 3 - Ungváry et. al. 1979

Test Substance	Ortho-xylene, (CAS # 95-47-6; meta-xylene, (CAS #108-38-3); para-xylene, (CAS #106-42-3); mixed xylenes = 1/3 each isomer, from Reachim. Soviet Union
Type (test type)	Acute Oral Toxicity - LD_{50}
Method/guideline followed	No guideline specified
GLP	No
Year	1979
Species/Strain	Rat/CFY
Sex	Not specified
No. of animals per dose	8
Vehicle	None
Route of administration	Oral
Test Conditions	Six groups (8 rats/group) were given a single oral dose of a xylene isomer or mixed isomer over a dose range with 1.3 grading [actual doses not specified]. Animals were observed for 14 days. LD_{50} values with confidence limits were calculated using the method of Behrens-Karber (1957). All animals that died or were killed on day 14 were necropsied and tissues from animals in the two highest dose groups were examined microscopically.
Results **LD_{50} with confidence limits**	o-xylene: 4.10 [3.57-4.71]ml/kg = 3.58g/kg (d=0.874) m-xylene: 5.80 [5.18-6.50]ml/kg = 5.01g/kg (d=0.864) p-xylene: 4.68 [3.94-5.55]ml/kg = 4.02g/kg (d=0.861) mixed: 6.72 [5.91-7.63]ml/kg = 5.83g/kg (based on avg of isomer d=0.867)
Remarks	Animals died of circulatory and respiratory failure, induced at different target points by each isomer. Gastrointestinal tract and bladder dilated; generalized bleeding in organs.
Conclusions (study author)	Ranking of acute oral toxicity of xylene isomers was o-xylene > p-xylene > m-xylene > mixed xylenes.
Data Quality Reliability	2. Reliable with restrictions. Actual doses not provided, sex of rats not specified.
References	Ungváry, G., Tátrai, E., Barcza, G., and Krasznai, G. 1979. Munkavédelem 25: 37-39 (English translation). Behrens-Karber, 1957. Toxicitási vizsgálatok: 854-862.

Acute Toxicity – Dermal

Acute Dermal Robust Summary 1 – Hine and Zuidema 1970

Test Substance	Mixed xylenes (C8 aromatics): ortho-, meta-, para-xylenes and ethyl benzene [percentages not reported]. Boiling range 138-141^{0}C [281-286^{0}F], 98% pure. From Shell Chemical Co., Petrochemical Division.
Method	
Method/guideline followed	Not specified. Standard procedure
Type (test type)	Dermal LD_{50}
GLP	No
Year	1970
Species/Strain	Rabbits, New Zealand White
Sex	Male [2-3kg]
No. of animals per sex per dose	3
Vehicle	None
Route of administration	Dermal
Test Conditions	Graded doses of mixed xylenes up to 5.0ml/kg were applied to intact skin on the backs of 3 shaved rabbits, occluded with a Saran-wrap sleeve. Contact was maintained for 4 hr, after which the covering was removed and skin wiped with a damp cloth. Rabbits were observed for 14 days after dosing for mortality and clinical signs of toxicity.
Results	LD_{50} = 5.0ml/kg = 4.3mg/kg
Remarks	One rabbit died on the 5th day after a dose of 5.0ml/kg. Survivors experienced discomfort and prostration during exposure but recovered. No deaths occurred at 2.0ml/kg =1.72mg/kg.
Conclusions (study author)	Mixed xylenes with an LD_{50} = 5.0ml/kg was considered practically non-toxic to the shaved skin of rabbits for 4hrs under occluded conditions.
Data Quality Reliability	**2**. Reliable with restrictions. Percentages of xylene isomers in the mixed xylene sample were not reported. This publication presented summarized results of tests with 4 aromatic and 6 aromatic-free solvents and did not report the actual range of doses tested.
	Hine, C.H., and Zuidema, H.H. 1970. The toxicological properties

References	of hydrocarbon solvents. Indust Med 39: 39-44.
Other **Last changed**	2/14/03

Acute Dermal Robust Summary 2 – Smyth et. al. 1962

Test Substance	Meta-xylene, commercial (CAS #108-38-3)
Type (test type)	Acute Dermal Toxicity - LD_{50}
Method/guideline followed	Standard procedure “akin to Draize one-day cuff method” [1944].
GLP	No
Year	1962 publication
Species/Strain	Rabbits/ New Zealand White
Sex	Male
No. of animals per sex per dose	4
Vehicle	None
Route of administration	Dermal application
Test Conditions	The fur of 4 male rabbits (2.5-3.5kg) was removed by clipping from the entire trunk. M-xylene in logarithmic doses (separation factor of 2) was applied to the skin and covered with an impervious plastic film. Rabbits were immobilized during the 24hr-exposure period, after which the occlusive cover was removed and animals were observed for 14 days. LD50 and fiducal limits were calculated by the method of Thompson [1947] using the tables of Weil [1952] with limits of ±1.96 standard deviations..
Results **LD_{50} with confidence limits.**	14.1ml/kg = 12.18g/kg.
Remarks	No fiducal range was calculated because no dose level resulted in fractional mortality. Data appeared in a table of

	more than 300 compounds, no additional specific information provided.
Conclusions (study author)	LD_{50} = 14.1ml/kg = 12.18g/kg.
Data Quality Reliability	2, Reliable with restrictions. No information was presented on m-xylene induced clinical signs, timing of deaths. Purity and source of m-xylene was not provided.
References	Smyth, H.F., Carpenter, C.P. Weil, C.S., Pozzani, U.C., and Striegel, J.A. 1962. Range-finding toxicity data: List VI. Amer Industr Hyg Assoc J 23: 95-107

Tier 1 Repeat Dose Toxicology

See Tier 2 Studies.

Tier 1 Genetic Toxicology – *In Vitro*

Tier 1: In Vitro Genetic Toxicity Robust Summary 1 - Bos et. al. 1981

Test Substance	0rtho-xylene (CAS #95-47-6); meta-xylene (CAS #108-38-3); para-xylene (CAS #106-42-3)
Type	Bacterial reverse mutation assay
Method/guideline followed	None specified. Standard method based on Ames et al., 1975
System of testing	Salmonella typhimurium with and without metabolic activation
GLP	No
Year	1981 publication
Species/Strain	Salmonella strains TA1535, TA1537, TA1538, TA98, TA100
Metabolic activation	Yes
Species and cell type	Liver S9 (9000G supernatant) from male Wistar rats
Quantity	0.1ml S9 supernatant/1ml S9 mix
Induced or not induced	Uninduced S9 and S9 from rats induced with Aroclor 1254 [dosage and treatment interval not specified]

Concentrations tested	0, (DMSO), 20, 50, 100, 200 and 500µg/plate diluted in dimethyl sufoxide (DMSO)
Statistical Methods	None specified.
Remarks for Test Conditions	Xylene isomers (o-, m-, p-) diluted in DMSO were tested individually in 5 strains of Salmonella with or without metabolic activation, 3 plates/dose level/strain. Metabolic activation was supplied by liver S9 from uninduced or Aroclor induced male Wistar rats. Although not described in detail in this report, the Ames assay (1975) is a plate incorporation test in which test material is introduced directly to histidine-deficient Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 (approx. 10^8cells/ml) ±S9 mix. After 48 hrs incubation at 37^0C, plates were counted with a Biotran II automated colony counter and results compared with negative and positive controls. Postive control compounds for non-activated plates were sodium azide (5µg/plate) positive for TA1535 and TA100; 4-nitroquinoline-1-oxide (2µg/plate), positive for TA1538, TA98 and TA100; 4-nitro-o-phenylenediamine (10µg/plate), positive for TA1538, TA98; 9-aminoacridine (200µg/plate), positive for TA1537. Positive control compounds for S9 activated plates were 2-aminoanthracene (1µg/plate) positive for all strains except TA1537; benzo(a)pyrene (7.5µg/plate), positive for all strains except TA1535; 9,10-dimethylbenzanthracene (20µg/plate) positive for TA1537, TA98, TA100.
Results **Genotoxic effects**	Xylene isomers (ortho-, meta-, para-) did not induce increases in mutant frequency in any strain of Salmonella with or without metabolic activation from induced or uninduced rat liver supernatant. Killing of bacteria was minimal; at the highest concentration [500µg/plate], 80-100% bacteria survived. Positive control compounds demonstrated appropriate mutagenic activity.
Conclusions (contractor)	Ortho-, meta-, and para-xylene are not gene mutagens in the Ames Salmonella assay with or without metabolic activation.
Data Quality Reliabilities	2, Reliable with restrictions. Assay performed according to standard method with appropriate number of replicates and adequate positive and negative controls. However, purity of test materials not specified.
Reference	Bos, R.P., Brouns, R.M.E., vanDoorn, R., Theuws, J.L.G., and Henderson, P. Th. 1981. Non-mutagenicity of toluene, o-, m-, and p-xylene, o-methylbenzylalcohol and o-methylbenzylsulfate in the Ames assay. Mutat. Res 88: 273-

279.

Ames, B.N. et al., 1975. Mutat. Res 31: 347-364.

Tier 1: In Vitro Genetic Toxicity Robust Summary 2 - Richer et. al. 1993

Test Substance
Test substance Xylene – mixture comprised of 15% ortho-, 25% para- and 60% meta-xylene isomers. Supplier not identified

Method
Method/guideline followed Guideline not specified
Type Sister chromatid exchange (SCE)
System of testing Human peripheral lymphocytes
GLP Not specified
Year 1993
Species/Strain Human (sex not provided) Adult, non-smoking
Metabolic activation NA
Species and cell type NA
Quantity NA
Induced or not induced NA
Concentrations tested 0, 50, 250, 500µM, 1.0, 2.0mM; in dimethyl sulfoxide [DMSO]
Statistical Methods Student's paired t-test (treated vs control), Analysis of variance when needed. P<0.05.

Remarks for Test Conditions Peripheral blood samples from healthy adult non-smoking volunteers [number, age, sex and race not specified], who had not previously been exposed to radiation, drug therapy or alcohol, were collected. Whole blood lymphocyte cultures were prepared by incubating 0.3ml heparinized whole blood in 5ml RPMI-1640 medium containing fetal calf serum and 1% phytohemaglutinin. Xylene was dissolved in DMSO (spectrograde) and added at concentrations from 50µM-2.0mM to cultures, which were incubated for 72hrs and 37^{0}C. No metabolic activation system was added because primary lymphocytes cultures have inherent metabolic capacity to convert aromatic hydrocarbons to active form. Whether or not negative control cultures were treated with DMSO or left untreated was not specified. No positive control compounds were reported. After 48hr, 10µM 5-bromo-deoxyuridine (BrdU) was added to label chromosome strands for SCE, and lymphocytes were harvested 24hr later. Two hours before harvest [46hr in BrdU], 0.1µg/ml colcemid was added to arrest cells division. After 72hr in culture, cells were treated with hypotonic [0.075M] KCl and fixed in absolute methanol/glacial acetic acid [3:1 v/v]. Slides were prepared by the air-drying method, stained by the fluorescene-photolysis-Giemsa method (Perry and Wolff, 1974) and coded for analysis. Slides were analyzed for cell mortality, cell cycle delay, and SCE formation. Forty metaphases in

the second round of replication from each preparation were scored for the number of SCE. Cell cycle delay was evaluated in 100 cells from each donor by measuring the percentage of mitoses in the first and second [and third] rounds of replication.

Results
Genotoxic effects

Dose-related increase in cell mortality was observed at all concentrations reaching approximately 50% at 2.0mM (estimated from figure). At concentrations of 50µM –1.0mM, there were no statistically significant effects on cell cycle kinetics; at 2.0mM, a significant delay in cell turnover rate was observed. An average value of 6.3 sister chromatid exchanges (SCE/cell) were counted in the 72hr lymphocyte cultures from control subjects, not exposed to any genotoxic substances. No significant effect on SCE incidence was observed at concentrations up to 250µM xylene. At higher concentrations, xylene induced a significant increase in SCE frequency, approximately 7.0, 7.5, 7.8 SCE/cell (estimated from figure, std. error 0.4-0.6) at 500µM, 1.0mM and 2.0mM, respectively.

Conclusions (contractor)

Xylene induced cell mortality over the range of doses tested that reached 50% at 2.0mM, the highest dose, however no significant effects on cell cycle were observed at any dose level except 2.0mM. Small but statistically significant increases in SCE (0.07-1.5 SCE/cell above controls) were observed at the three highest doses of xylene [500µM-2.0mM]. The authors concluded that “exposure of human blood lymphocytes *in vitro* to xylene did not result in significant effects on the cytogenetic parameters tested [SCE and cell cycle kinetics] at lower, minimally toxic concentrations; at higher concentrations, only cell mortality was significantly affected.”

Data Quality
Reliabilities

2. Reliable with restrictions. Treatment of negative control cultures was not specified. No actual data were provided; numerical values were estimated from histograms. No positive control compounds were used. Comparison with positive control data for SCE would more strongly support the authors’ conclusions that the small increases in SCE at higher concentrations were not toxicologically significant.

Reference

Richer C.-L., Chakrabarti, S., Senécal-Quevillon, M., Duhr, M.A., Zhang, X.X., and Tardiff, R. 1993. Cytogenetic effects of low-level exposure to toluene, xylene, and their mixture on human blood lymphocytes. Int Arch Occup Environ Health 64: 581-585. [See robust summary on *in vivo* exposure study]
Perry, P., and Wolff, S. 1974. Nature 258: 121-125.

Other
Last changed

2/14/2003

Xylene VCCEP Robust Summaries

Tier 2 Studies

Tier 2 Subchronic Toxicity

Tier 2: Subchronic Toxicity Robust Summary 1a (m-xylene) - Wolfe 1988

Test Substance Remarks	Meta-xylene (CAS #108-38-3); clear, colorless liquid from Fluka Chemical Corp., Hauppauge, NY. 99% pure. Material Safety Data Sheet provided.
Method/guideline followed	"Standard EPA guidelines" reported.
Test type	Subchronic oral
GLP	Yes
Year	1988
Species/Strain	Rat / Sprague Dawley from Charles River Labs., Portage, Mich
Route of administration	Oral gavage
Duration of test	13 weeks
Doses/concentration levels	0, 100, 200, and 800mg/kg/day
Sex	Male and female [20/sex/group]
Exposure period	90 days
Frequency of treatment	once/day
Control group and treatment	20M, 20F rats, corn oil [2.5ml/kg]
Post exposure observation period	None
Statistical methods	Cumulative survival data analyzed by Nat'l Cancer Institute package (Life Table analysis). Body wt, food consumption, clinical pathology, organ wt data evaluated by Levene's test of homogeneity of variances, ANOVA (5% one-tailed probability), Dunnett's t-test (5% 2-tailed). For heterogeneous variances, appropriate data transformations were performed.
Test Conditions	Sprague Dawley rats (20M, 20F/group; 46 days old at study initiation) had been acclimated for 2 wks prior to assignment to dose groups. Body wts at initiation were 203.7-246.4g for males; 142.1-174.8g for females. Rats, uniquely identified by ear tags and cage cards, were housed individually in elevated wire-mesh cages in a room maintained at 70-77^{0}F and 31-69% relative humidity with a 12 hr light-dark cycle. Food (Purina certified rodent chow) and acidified tap water were available ad lib [contaminant analyses provided]. Animals were assigned to treatment groups using a computer

weight randomization program. A baseline group (5M, 5F) was used for pretreatment blood work then discarded without necropsy. Neat m-xylene was stored frozen and protected from light. Formulations for each dose level were prepared weekly in corn oil volumetrically, adjusted to 100% activity based on 99% purity. Formulated material was stored at room temperature. Analysis of neat m-xylene to verify identity and purity were performed prior to initiation and for purity only at termination by IR spectra, MNR, GC/MS, GC purity, and elemental analysis. m-Xylene formulated in corn oil was analyzed by GC/FID. Stability of high (323.2mg/ml) and low (40.4mg/ml) dose concentrations were determined after 7, 14 and 21 days storage at room temp and at 5^0C. Dose verification analyses of corn oil formulations were performed at wk 1, 5, 9, and 13 of study. Each batch of corn oil was analyzed for peroxides prior to first use and at monthly intervals [Appendix 9]. All rats were observed for overt signs of toxicity daily, mortality/moribundity twice a day, and complete physical exams were performed at initiation and weekly thereafter. Body wt was recorded during randomization, at initiation, and weekly during the study. Food consumption was recorded weekly. Opthalmoscopic examinations were performed prior to treatment, and on the last 10 surviving animals/sex/group during wk 13. Clinical pathology determinations were made on 5 rats/sex [baseline group] prior to initiation and on the first 10 surviving rats/sex/group during wk 5 and 13. Animals were fasted overnight and samples collected by orbital sinus bleeding under CO_2 anesthesia. Hematology parameters were RBC and WBC counts, hemoglobin, hematocrit, platelets, differential leukocytes, reticulocyte counts, and cell morphology. Serum chemistries were sodium, potassium, calcium, chloride, total protein, albumin, phosphorus, total bilirubin, urea nitrogen, creatinine, glucose, SGOT/AST, SGPT/ALT, globulin, alkaline phosphatase, cholesterol, albumin/globulin ratio, and lactate dehydrogenase. All rats that died during the study and animals at terminal sacrifice were given a complete gross necropsy. Animals were killed by exsanguination under sodium pentabarbital anesthesia. Five animals/sex from control and 10 animals/sex/treatment group were selected for electron microscopy (EM results were not presented in this study). Organs weighed for all animals except EM rats were liver, kidneys, spleen, adrenal glands, brain with stem, heart, testes with epididymides, and ovaries. Organ to terminal body wt ratios (relative organ wt) were calculated. Thirty-seven tissues and all gross lesions were preserved in 10% formalin, embedded, sectioned and stained with hematoxylin and eosin. All tissues from control

and 800mg/kg/day rats, any rats dying prior to study termination, and gross lesions, lung, liver and kidneys from other dose groups were examined microscopically.

Results
NOAEL (NOEL)
LOAEL (LOEL)

Females
NOEL = 200mg/kg; LOEL = 800mg/kg [based on decreased body wt]
Males
NOEL = 100mg/kg; LOEL = 200mg/kg [based on decreased body wt]

Remarks

Analytical: Stability of m-xylene in corn oil stored for 21 days showed a mean loss of 2.8% at 5^0C and 1.7% at room temperature. Dose verification results were within ±10% of target concentrations. Peroxide numbers for corn oil batches were within acceptable limits [<10meq/kg].
Mortality: Unscheduled deaths occurred in 4F, 2M; 5F, 5M, and 0F, 3M at the 800, 200 and 100mg/kg/day treatment levels, respectively; no early deaths were reported in controls. In females, mortality showed a statistically significant trend but not in males. Histopathology indicated foreign material in alveoli of the majority of animals that elicited little or no inflammatory reaction except for one 800mg/kg male who developed foreign body pneumonia. There was no physical evidence of trauma [perforated trachea and/or esophagus. These deaths were considered to be due to aspiration of dosing formulation, related to possible gavage accidents and the low vapor pressure of xylene [~10mm Hg at 28^0C].
Clinical signs: Chromodacryorrhea, and malocclusion in all groups and urine stains in high dose animals only were commonly observed weekly. Occurrences of hyperactivity, convulsions, salivation and epistaxis were observed during the first 3 weeks of study with low frequency. Only salivation persisted in both sexes at 800mg/kg throughout the study with higher incidence in females; animals returned to normal by the one hour post-dose observation. No ocular abnormalities were seen at terminal examination.
Body Wt/ Food consumption: Mean body wt gain was significantly decreased in males given 200 or 800mg/kg/day and in 800mg/kg/day females, showing a clear relationship between dose and body wt depression. Food consumption calculated over wks 1-5, wks 6-9, and wks 10-13, was decreased in 800mg/kg/day males in wks 1-5 and 6-9, and in 200mg/kg/day males in wks 6-9. Female food consumption was comparable to controls.
Clinical Pathology: At wk 5, hematocrit decreased, cholesterol, calcium, and potassium increased in 800mg/kg/day females and potassium also increased in 200mg/kg/day females. Potassium increased and total

	bilirubin decreased in 800mg/kg/day males. There were fewer significant changes at wk 13 – alanine aminotransferase (ALT) increased in 800mg/kg/day males, and calcium and cholesterol increased in 800mg/kg/day females. Organ wts/Pathology: No significant changes were observed in females. In 800mg/kg/day males, heart wt decreased and relative brain and kidney wts were increased. Most common lesions at necropsy were mottled lungs or lungs that failed to collapse, found only in animals found dead. No significant lesions were observed by gross examination. At histopathology spontaneously occurring lesions were observed of a type and frequency expected for this strain and age of rat; one 100mg/kg/day female had a mammary adenocarcinoma unrelated to treatment.
Conclusions (study authors)	m-Xylene induced very few toxic effects in rats treated by gavage at doses up to 800mg/kg/day for 90 days. NOEL and LOEL were assigned based on decreased body wt, and with consideration of significant increased mortality, attributed to aspiration of dosing formulation at intubation. Few clinical pathology parameters, primarily at 800mg/kg, showed increases or decreases. In high dose males, absolute heart wt decreased and relative brain and kidney wt increased. Histopathology findings were unremarkable.
Quality Reliabilities	1, Reliable without restrictions
References	Wolfe, G.W. 1988. Subchronic toxicity study in rats with m-xylene. HLA Proj. #2399-108 Hazleton Laboratories America, Inc. Rockville, MD. performed for Dynamac Corp., Rockville, MD

Tier 2: Subchronic Toxicity Robust Summary 1b (p-xylene) - Wolfe 1988

Test Substance Remarks	Para-xylene (CAS #106-42-3); clear, colorless liquid from Fluka Chemical Corp., Hauppauge, NY. 99% pure. Material Safety Data Sheet provided.
Test type	Subchronic oral
Method/guideline followed	“Standard EPA guidelines” reported
GLP	Yes
Year	1988
Species/Strain	Rat / Sprague Dawley from Charles River Labs., Portage, Mich
Route of administration	Oral gavage
Duration of test	13 weeks

Doses/concentration levels	0, 100, 200, and 800mg/kg/day
Sex	Male and female [20/sex/group]
Exposure period	90 days
Frequency of treatment	once/day
Control group and treatment	20M, 20F rats, corn oil [2.5ml/kg]
Post exposure observation period	None
Statistical methods	Cumulative survival data analyzed by Nat'l Cancer Institute package (Life Table analysis). Body wt, food consumption, clinical pathology, organ wt data evaluated by Levene's test of homogeneity of variances, ANOVA (5% one-tailed probability), Dunnett's t-test (5% 2-tailed). For heterogeneous variances, appropriate data transformations were performed.
Test Conditions	Sprague Dawley rats (20M, 20F/group; 45 days old at study initiation) had been acclimated for approx. 2 wks prior to assignment to dose groups. Body wts at initiation were 199.1-257.8g for males; 144.9-192.1g for females. Rats, uniquely identified by ear tags and cage cards, were housed individually in elevated wire-mesh cages in a room maintained at 71-78^{0}F and 31-69% relative humidity with a 12 hr light-dark cycle. Food (Purina certified rodent chow) and acidified tap water were available ad lib [contaminant analyses provided]. Animals were assigned to treatment groups using a computer weight randomization program. A baseline group (5M, 5F) was used for pretreatment blood work then discarded without necropsy. Neat p-xylene was stored frozen and protected from light. Formulations for each dose level were prepared weekly in corn oil volumetrically, adjusted to 100% activity based on 99% purity. Formulated material was stored at room temperature. Analysis of neat p-xylene to verify identity and purity were performed prior to initiation and for purity only at termination by IR spectra, MNR, GC/MS, GC purity, and elemental analysis. p-Xylene formulated in corn oil was analyzed by GC/FID. Stability of high (323.2mg/ml) and low (40.4mg/ml) dose concentrations were determined after 7, 14 and 21 days storage at room temp and at 5^{0}C. Dose verification analyses of corn oil formulations were performed at wk 1, 5, 9, and 13 of study. Each batch of corn oil was analyzed for peroxides prior to first use and at monthly intervals [Appendix 9]. All rats were observed for overt signs of toxicity daily, mortality/moribundity twice a day, and complete physical exams were performed at initiation and weekly thereafter. Body wt was recorded during randomization, at initiation, and weekly during the study. Food consumption was recorded weekly. Opthalmoscopic

examinations were performed prior to treatment, and on the last 10 surviving animals/sex/group during wk 13. Clinical pathology determinations were made on 5 rats/sex [baseline group] prior to initiation and on the first 10 surviving rats/sex/group during wk 5 and 13. Animals were fasted overnight and samples collected by orbital sinus bleeding under CO_2 anesthesia. Hematology parameters were RBC and WBC counts, hemoglobin, hematocrit, platelets, differential leukocytes, reticulocyte counts, and cell morphology. Serum chemistries were sodium, potassium, calcium, chloride, total protein, albumin, phosphorus, total bilirubin, urea nitrogen, creatinine, glucose, SGOT/AST, SGPT/ALT, globulin, alkaline phosphatase, cholesterol, albumin/globulin ratio, and lactate dehydrogenase. All rats that died during the study and animals at terminal sacrifice were given a complete gross necropsy. Animals were killed by exsanguination under sodium pentabarbital anesthesia. Five animals/sex from control and 10animals/sex/treatment group were selected for electron microscopy (EM results were not presented in this study). Organs weighed for all animals except EM rats were liver, kidneys, spleen, adrenal glands, brain with stem, heart, testes with epididymides, and ovaries. Organ to terminal body wt ratios (relative organ wt) were calculated. Thirty-seven tissues and all gross lesions were preserved in 10% formalin, embedded, sectioned and stained with hematoxylin and eosin. All tissues from control and 800mg/kg/day rats, any rats dying prior to study termination, and gross lesions, lung, liver and kidneys from other dose groups were examined microscopically

Results
NOAEL (NOEL)
LOAEL (LOEL)
Remarks

NOEL = 200mg/kg; LOEL = 800mg/kg for both sexes.

Analytical: Stability of p-xylene in corn oil stored for 21 days showed a mean loss of <1.0% at room temperature and no loss of sample stored at 5^0C. Dose verification results were within ±10% of target concentrations. Peroxide numbers for corn oil batches were within acceptable limits [<10meq/kg].

Mortality: Unscheduled deaths occurred in 3F, 4M; 2F, 3M, and 2F, 1M at the 800, 200 and 100mg/kg/day treatment levels, respectively; no early deaths were reported in controls. Significantly higher mortality occurred in 800mg/kg/day males and a significant trend for increased mortality in males was observed. There were no significant differences in female mortality and no significant trend over dose groups was observed. Intra-alveolar foreign material was present in lungs of nearly all unscheduled deaths. This finding was often associated with congestion, and appeared secondary to aspiration of dosage formulation, related to possible gavage

accidents and the low vapor pressure of xylene [~10mm Hg at 28^0C]. There was no physical evidence of trauma [perforated trachea and/or esophagus].

Clinical signs: Most commonly observed weekly symptoms were chromodacryorrhea, malocclusion and sores in all groups and salivation in high dose animals only. Salivation persisted in both sexes at 800mg/kg throughout the study from the 2nd week of treatment, beginning just prior to dosing; animals returned to normal by the one hour post-dose observation. No ocular abnormalities were seen at terminal examination.

Body Wt/ Food consumption: Mean body wt of both sexes in the 800mg/kg/day group was lower than other groups but not statistically significant at wk 13. Although wk 0-13 body wt gain in high dose rats was less than controls, the decrement was not statistically significant [Note: By the end of wk 13, the high dose groups was comprised of 16M, 17F compared to 20M, 20F in controls.] The 100 and 200mg/kg/day groups had similar or slightly higher wt gain compared to controls. Food consumption calculated over wk 1-5, wk 6-9, and wk 10-13, was highest in the top dose group of both sexes; significantly increased in females in wk 10-13.

Clinical Pathology: There were no significant differences in any hematology parameters at wk5 but 800mg/kg/day female serum chemistries showed significant increases in phosphorus, cholesterol and alanine aminotransferase (ALT) that were not present at wk 13. At wk 13, 200mg/kg/day males had a significant decrease in segmented neutrophils, but there were no significant serum chemistry changes for either sex at any dose level.

Organ Wt/Pathology: No significant changes between control and treated animals of either sex were observed for absolute or relative organ wts. No significant lesions were seen at gross examination. Mottled lungs and lungs that failed to collapse occurred only in animals that died prior to study termination. There were no specific test material related histopathological lesions. In both treated and control rats, lesions present in the liver were monoclonal cell infiltrates, subacute inflammation and fatty changes; in kidneys, lesions consisted of tubular regeneration, focal mononuclear cell infiltrates, and interstitial fibrosis. Intra-alveolar foreign material in lungs of animals found dead resulted from aspiration of dosing formulation.

Conclusions

Para-xylene induced very few toxic effects in rats treated by

(study authors)	gavage at doses up to 800mg/kg/day. NOEL was selected in consideration of slight mean body wt decreases in high dose animals, and increased mortality, statistically significant in 800mg/kg/day males, correlated with dosing formulation aspiration. Opthalmology, organ wt, gross pathology, and histopathology findings were unremarkable.
Quality Reliabilities	1, Reliable without restrictions
References	Wolfe, G.W. 1988. Subchronic toxicity study in rats with p-xylene. HLA Proj. #2399-110 Hazleton Laboratories America, Inc. Rockville, MD, performed for Dynamac Corp., Rockville, MD.

Tier 2: Subchronic Toxicity Robust Summary 2a - National Toxicology Program (NTP) (mice) 1986

Test Substance Remarks	Mixed xylenes, CAS #1330-20-7; 60% meta-, 14% para-, (% ortho-xylene, and 17% ethyl benzene. Mol wt. 106.2. from Shell Oil Co, Houston, TX. Purity and composition determined by elemental analysis and infrared, UV/visible, and nuclear magnetic resonance analysis.
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Subchronic – 13wk oral
GLP	Yes
Year	1986
Species/Strain	Mice, B6C3F1
Route of administration	Oral, gavage
Duration of test	13 weeks
Doses/concentration levels	0, 125, 250, 500, 1000, and 2000mg/kg in corn oil
Sex	Male and female (10/sex/group)
Exposure period	13 weeks
Frequency of treatment	once daily, 5 days/wk
Control group and treatment	10M, 10F; corn oil (8ml/kg)

Post exposure observation period

None

Statistical methods

None specified

Test Conditions

Male and female mice (4wk old at receipt) from Charles River Breeding Laboratories were observed for 2 weeks. At the end of the quarantine period, mice were individually weighed to determine the weight range for each sex, then distributed by weight class into polycarbonate cages with polyester filters, 5 rats/cage. Cages were assigned to test groups according to tables of random numbers. Animals were weighed, identified by toe clip, and given food [Purina Lab Chow- analysis supplied] and water *ad libitum*. Animal rooms were maintained at 22±1^{0}C, 40-60% relative humidity with 15 room air changes per hour, and 12hr light/dark cycle. Mixed xylene in corn oil was administered in doses of 0, 125, 250, 500, 1000 and 2000mg/kg/day, 5 days/wk for 13 weeks. Animals were observed twice daily for mortality and moribundity, and clinical signs. Body weights were recorded weekly. Food consumption was not recorded. At the end of 13 weeks, surviving mice were killed and necropsies were performed on all animals including those found dead unless they were excessively autolyzed or cannibalized. Organs were not weighed. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and slides stained with hematoxylin-eosin. Tissues from control and high dose mice examined histopathologically included gross lesions and tissue masses, mandibular lymph nodes, salivary glands, sternebrae, femur or vertebrae including marrow, thyroid, parathyroids, small intestines, colon, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, liver, gall bladder, prostate/testes, ovaries/uterus, mammary gland, lungs and main stem bronchi, adrenals, urinary bladder, pituitary, spinal cord [if neurological signs present], and eyes[if grossly abnormal].

Results
NOAEL (NOEL)
LOAEL (LOEL)

NOAEL = 1000mg/kg

LOAEL = 2000mg/kg [deaths, reduced body wt gain, clinical signs]

Remarks

Two female mice given 2000mg/kg/day died in wk5 and wk 10, respectively. Weakness, lethargy, short and shallow breathing, unsteadiness, tremors and paresis were observed in 2000mg/kg mice 5-10min after dosing, lasting 15-60min. Mean body wt gain in high dose mice was 5% and 17% lower than controls for males and females

	respectively. No compound-related gross or microscopic pathological lesions were observed.
Conclusions (study authors)	Administration of mixed xylenes orally to mice at 2000mg/kg/day for 13wk resulted in mortality [2F], clinical signs lasting up to 1hr after dosing, and reduced body wt gain. [particularly in females] but did not cause overt signs of toxicity or pathological changes. This study, in conjunction with an earlier 14-day study was used to establish doses for a 2-year cancer bioassay.
Quality Reliabilities	2. Reliable with restrictions. Data presented is adequate, but endpoints are incomplete since it was performed as a range-finding study for a 2-year assay.
References	National Toxicology Program. 1986. Toxicology and carcinogenesis studies of xylene (mixed) in F344/N rats and B6C3F1 mice (gavage studies). Performed at Batelle Columbus, OH. Technical Report #327. National Institutes of Health, US DHHS, Washington, DC
Other Last changed	2/14/2003

Tier 2: Subchronic Toxicity Robust Summary 2b - National Toxicology Program (NTP) (rats) 1986

Test Substance Remarks	Mixed xylenes, CAS #1330-20-7; 60% meta-, 14% para-, (% ortho-xylene, and 17% ethyl benzene. Mol wt. 106.2. from Shell Oil Co, Houston, TX. Purity and composition determined by elemental analysis and infrared, UV/visible, and nuclear magnetic resonance analysis.
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Subchronic – 13wk oral
GLP	Yes
Year	1986
Species/Strain	Rats F344/N
Route of administration	Oral, gavage

Duration of test	13 weeks
Doses/concentration levels	0, 62.5, 125, 250, 500, and 1000mg/kg in corn oil
Sex	Male and female (10/sex/group)
Exposure period	13 weeks
Frequency of treatment	once daily, 5 days/wk
Control group and treatment	10M, 10F; corn oil (4ml/kg)
Post exposure observation period	None
Statistical methods	None specified
Test Conditions	Male and female rats (4wk old at receipt) from Charles River Breeding Laboratories were observed for 2 weeks. At the end of the quarantine period, rats were individually weighed to determine the weight range for each sex, then distributed by weight class into polycarbonate cages with polyester filters, 5 rats/cage. Cages were assigned to test groups according to tables of random numbers. Animals were weighed, identified by toe clip, and given food [Purina Lab Chow- analysis supplied] and water *ad libitum*. Animal rooms were maintained at 22±1^{0}C, 40-60% relative humidity with 15 room air changes per hour, and 12hr light/dark cycle. Mixed xylene in corn oil was administered in doses of 0, 62.5, 125, 250, 500, and 1000mg/kg/day, 5 days/wk for 13 weeks. Animals were observed twice daily for mortality and moribundity, and clinical signs. Body weights were recorded weekly. Food consumption was not recorded. At the end of 13 weeks, surviving rats were killed and necropsies were performed on all animals including those found dead unless they were excessively autolyzed or cannibalized. Organs were not weighed. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and slides stained with hematoxylin-eosin. Tissues from control and high dose rats examined histopathologically included gross lesions and tissue masses, mandibular lymph nodes, salivary glands, sternebrae, femur or vertebrae including marrow, thyroid, parathyroids, small intestines, colon, heart, esophagus, stomach, brain, thymus, trachea, pancreas,

	spleen, kidneys, liver, prostate/testes, ovaries/uterus, mammary gland, lungs and main stem bronchi, adrenals, urinary bladder, pituitary, spinal cord [if neurological signs present], and eyes[if grossly abnormal].
Results NOAEL (NOEL) LOAEL (LOEL)	NOAEL = 500mg/kg LOAEL = 1000mg/kg [reduced body wt gain]
Remarks	All rats survived to the end of the study. Mean body wt gain of male and female rats given 1000mg/kg/day were 15% and 8% lower than vehicle controls, respectively. No overt signs of toxicity were seen and no compound-related gross or macroscopic pathological lesions were observed.
Conclusions (study authors)	Administration of mixed xylenes orally at 1000mg/kg/day for 13wk resulted in reduced body wt gain in rats [particularly males] but did not cause overt signs of toxicity or pathological changes. This study, in conjunction with an earlier 14-day study was used to establish doses for a 2-year cancer bioassay.
Quality Reliabilities	2. Reliable with restrictions. Data presented is adequate, but endpoints incomplete since it was performed as a range-finding study for a 2-year assay.
References	National Toxicology Program. 1986. Toxicology and carcinogenesis studies of xylene (mixed) in F344/N rats and B6C3F1 mice (gavage studies). Performed at Batelle Columbus, OH. Technical Report #327. National Institutes of Health, US DHHS, Washington, DC
Other Last changed	2/14/2003

<u>Tier 2 – In Vivo Genetic Toxicity</u>

<u>Tier 2: In Vivo Genetic Toxicity Robust Summary 1a (m-xylene) – Mohtashamipur et. al. 1985</u>

Test Substance Remarks	Meta-xylene, CAS #108-38-3, 98% pure from Merck Co. (D-8011 Hohenbrunn, FRG) d=0.864
Type	Micronucleus assay
Method/guideline followed	Method of Schmid (1977)

GLP	Not specified
Year	1985
Species/Strain	Mice/NMRI
Sex	Male – 5/group
Route of administration	Intraperitoneal [IP]
Doses/concentration levels	0, 0.37, 0.50, 0.62, and 0.75ml/kg/dose [0, 0.32, 0.43, 0.54, and 0.65g/kg/dose]
Exposure period	30hr [2 doses, 24 hr apart]
Control/ treatment	10 male mice; corn oil IP
Statistical methods	Student's t-test (Hill, 1967)
Remarks for Test Conditions.	Each group of 5 male mice (25-30g) was administered one dose of m-xylene IP 24 hours prior to the second dose. Mice were housed 5/group. The combined maximum dose did not exceed 70% of a previously determined LD_{50}=2.00ml/kg. Lowest doses were given in corn oil for ease of injection. Six hours after the second dose [30hr after the first dose], animals were killed by ether overdose, both femora were removed, marrow was aspirated and suspended in fetal calf serum. The suspension was centrifuged and sedimented cells were used to prepare smears (2 smears/femur), which were fixed and stained (stain nor specified). Slides were coded and evaluated under high magnification by examiners without knowledge of treatment and doses. One thousand polychromatic erythrocytes/smear were screened for micronuclei. Positive control compounds were benzene (0.15, 0.30, 0.60ml/kg/dose), cyclophosphamide (0.05, 0.25ml/kg/dose) and 4-nitroquinolin-1-oxide (0.12, 0.25, 0.50ml/kg/dose). In addition, a random selection of smears from treated mice were studied double-blind by 2 different examiners to confirm accuracy of results.
Results	Negative.
Genotoxic effects: NOAEL (NOEL) LOAEL (LOEL)	None. NOEL = 0.75ml/kg/dose [0.65g/kg/dose] maximum dose tested. Meta-xylene did not induce increased incidence of micronuclei (MN) in polychromatic erythrocytes (PCE) in bone marrow lymphocytes of treated mice. Incidence of micronuclei/1000PCE was 2.0, 1.6, 1.9, and 1.9 at doses of 0.37, 0.50, 0.62, or 0.75ml/kg/dose respectively, compared to a negative control value of 1.9 MN/1000PCE. Counts of mature

normochromatic erythrocytes and the ratio of PCE to total erythrocytes [PCE + normochromatic erythrocytes (NCE)] were not calculated. Overt toxicity to bone marrow or the animals overall was not observed. Positive control compounds induced expected increases in frequency of micronuclei. At the maximum dose tested, benzene induced 12.9 MN/1000PCE, cyclophosphamide induced 21.1 MN/1000PCE, and 4-nitroquinolin-1-oxide induced 18.0 MN/1000PCE.

Conclusions (study authors)

Meta-xylene did not induce increased frequencies of micronucleated polychromatic erythrocytes in femoral bone marrow of treated mice, and is not a clastogen in this test system.

Data Quality Reliabilities

2. Reliable with restrictions. The ratio of polychromatic (immature) erythrocytes to total erythrocytes was not determined. The biological stain used to visualize micronuclei was not reported.

References

Mohtashamipur, E., Norpoth, K., Woelke, U., and Huber, P. 1985. Effects of ethylbenzene, toluene, and xylene on the induction of micronuclei in bone marrow polychromatic erythrocytes of mice. Arch Toxicol 58: 106-109.
Schmid W. 1977. in Kilbey et al., Eds. Handbook for mutagenicity test procedures. Elsevier, NY. pp. 235-242.
Hill, A.B. 1967 in Hill, A.B. Ed., Principles of Medical Statistics. The Lancet Ltd., London, England. pp. 146-151

Tier 2: In Vivo Genetic Toxicity Robust Summary 1b (o-xylene) – Mohtashamipur et. al. 1985

Test Substance Remarks

Ortho-xylene, CAS #95-47-6, 98% pure from Merck Co. (D-8011 Hohenbrunn, FRG) d=0.874

Method

Method/guideline followed

Method of Schmid (1977)

Type

Micronucleus assay

GLP

Not specified

Year

1985

Species

Mice

Strain

NMRI

Sex

Male – 5/group

Route of

Intraperitoneal [IP]

administration	
Doses/concentration levels	0, 0.12, 0.25, 0.37, and 0.50ml/kg/dose [0, 0.10, 0.22, 0.32, and 0.44g/kg/dose]
Exposure period	30hr [2 doses, 24 hr apart]
Control/ treatment	10 male mice; corn oil IP
Statistical methods	Student's t-test (Hill, 1967)
Remarks for Test Conditions.	Each group of 5 male mice (25-30g) was administered one dose of o-xylene IP 24 hours prior to the second dose. Mice were housed 5/group. The combined maximum dose did not exceed 70% of a previously determined LD_{50}=1.55ml/kg. Lowest doses were given in corn oil for ease of injection. Six hours after the second dose [30hr after the first dose], animals were killed by ether overdose, both femora were removed, marrow was aspirated and suspended in fetal calf serum. The suspension was centrifuged and sedimented cells were used to prepare smears (2 smears/femur), which were fixed and stained (stain nor specified). Slides were coded and evaluated under high magnification by examiners without knowledge of treatment and doses. One thousand polychromatic erythrocytes/smear were screened for micronuclei. Positive control compounds were benzene (0.15, 0.30, 0.60ml/kg/dose), cyclophosphamide (0.05, 0.25ml/kg/dose) and 4-nitroquinolin-1-oxide (0.12, 0.25, 0.50ml/kg/dose). In addition, a random selection of smears from treated mice were studied double-blind by 2 different examiners to confirm accuracy of results.
Results **Genotoxic effects** **NOAEL (NOEL)** **LOAEL (LOEL)**	NOEL = 0.50ml/kg/dose [0.44g/kg/dose] maximum dose tested Ortho-xylene did not induce increased incidence of micronuclei (MN) in polychromatic erythrocytes (PCE) in bone marrow lymphocytes of treated mice. Incidence of micronuclei/1000PCE was 1.80, 1.60, 1.70, and 1.80at doses of 0.10, 0.22, 0.32, or 0.50ml/kg/dose respectively, compared to a negative control value of 1.9 MN/1000PCE. Counts of mature normochromatic erythrocytes and the ratio of PCE to total erythrocytes [PCE + normochromatic erythrocytes (NCE)] were not calculated. Overt toxicity to bone marrow or the animals overall was not observed. Positive control compounds induced expected increases in frequency of micronuclei. At the maximum dose tested, benzene induced 12.9 MN/1000PCE, cyclophosphamide induced 21.1 MN/1000PCE, and 4-nitroquinolin-1-oxide induced 18.0 MN/1000PCE.
Conclusions	Ortho-xylene did not induce increased frequencies of

(study authors)	micronucleated polychromatic erythrocytes in femoral bone marrow of treated mice, and is not a clastogen in this test system.
Data Quality Reliabilities	**2.** Reliable with restrictions. The ratio of polychromatic (immature) erythrocytes to total erythrocytes was not determined. The biological stain used to visualize micronuclei was not reported.
References	Mohtashamipur, E., Norpoth, K., Woelke, U., and Huber, P. 1985. Effects of ethylbenzene, toluene, and xylene on the induction of micronuclei in bone marrow polychromatic erythrocytes of mice. Arch Toxicol 58: 106-109. Schmid W. 1977. in Kilbey et al., Eds. Handbook for mutagenicity test procedures. Elsevier, NY. pp. 235-242. Hill, A.B. 1967 in Hill, A.B. Ed., Principles of Medical Statistics. The Lancet Ltd., London, England. pp. 146-151
Other	
Last changed	2/05/2003

Tier 2: In Vivo Genetic Toxicity Robust Summary 1c (p-xylene) – Mohtashamipur et. al. 1985

Test Substance Remarks	Para-xylene, CAS #106-42-3, 98% pure from Merck Co. (D-8011 Hohenbrunn, FRG) d=0.861
Method	
Method/guideline followed	Method of Schmid (1977)
Type	Micronucleus assay
GLP	Not specified
Year	1985
Species	Mice
Strain	NMRI
Sex	Male – 5/group
Route of administration	Intraperitoneal [IP]
Doses/concentration levels	0, 0.37, 0.50, 0.62, and 0.75ml/kg/dose [0, 0.32, 0.43, 0.53, and 0.65g/kg/dose]

Exposure period	30hr [2 doses, 24 hr apart]
Control/ treatment	10 male mice; corn oil IP
Statistical methods	Student's t-test (Hill, 1967)
Remarks for Test Conditions.	Each group of 5 male mice (25-30g) was administered one dose of p-xylene IP 24 hours prior to the second dose. Mice were housed 5/group. The combined maximum dose did not exceed 70% of a previously determined LD_{50}=2.45ml/kg. Lowest doses were given in corn oil for ease of injection. Six hours after the second dose [30hr after the first dose], animals were killed by ether overdose, both femora were removed, marrow was aspirated and suspended in fetal calf serum. The suspension was centrifuged and sedimented cells were used to prepare smears (2 smears/femur), which were fixed and stained (stain nor specified). Slides were coded and evaluated under high magnification by examiners without knowledge of treatment and doses. One thousand polychromatic erythrocytes/smear were screened for micronuclei. Positive control compounds were benzene (0.15, 0.30, 0.60ml/kg/dose), cyclophosphamide (0.05, 0.25ml/kg/dose) and 4-nitroquinolin-1-oxide (0.12, 0.25, 0.50ml/kg/dose). In addition, a random selection of smears from treated mice were studied double-blind by 2 different examiners to confirm accuracy of results.
Results **Genotoxic effects** **NOAEL (NOEL)** **LOAEL (LOEL)**	NOEL = 0.75ml/kg/dose [0.65g/kg/dose] maximum dose tested Para-xylene did not induce statistically significant increased incidences of micronuclei (MN) in polychromatic erythrocytes (PCE) in bone marrow lymphocytes of treated mice. Incidence of micronuclei/1000PCE was 1.90, 1.90, 2.25, 2.40 at doses of 0.37, 0.50, 0.62, or 0.75ml/kg/dose respectively, compared to a negative control value of 1.9 MN/1000PCE. Although a slight, dose-related trend in increased frequency of micronucleated polychromatic erythrocytes seemed apparent, statistical significance was not reached compared to negative controls, and values were well below increases seen with positive control compounds. Counts of mature normochromatic erythrocytes and the ratio of PCE to total erythrocytes [PCE + normochromatic erythrocytes (NCE)] were not calculated. Overt toxicity to bone marrow or the animals overall was not observed. Positive control compounds induced expected increases in frequency of micronuclei. At the maximum dose tested, benzene induced 12.9 MN/1000PCE, cyclophosphamide induced 21.1 MN/1000PCE, and 4-nitroquinolin-1-oxide induced 18.0 MN/1000PCE.

Conclusions (study authors)	Para-xylene did not induce increased frequencies of micronucleated polychromatic erythrocytes in femoral bone marrow of treated mice, and is not a clastogen in this test system.
Data Quality Reliabilities	**2.** Reliable with restrictions. The ratio of polychromatic (immature) erythrocytes to total erythrocytes was not determined. The biological stain used to visualize micronuclei was not reported.
References	Mohtashamipur, E., Norpoth, K., Woelke, U., and Huber, P. 1985. Effects of ethylbenzene, toluene, and xylene on the induction of micronuclei in bone marrow polychromatic erythrocytes of mice. Arch Toxicol 58: 106-109. Schmid W. 1977. in Kilbey et al., Eds. Handbook for mutagenicity test procedures. Elsevier, NY. pp. 235-242. Hill, A.B. 1967 in Hill, A.B. Ed., Principles of Medical Statistics. The Lancet Ltd., London, England. pp. 146-151
Other	
Last changed	2/05/2003

Tier 2 In Vivo Genetic Toxicity Robust Summary 2 - Richer (1993)

Test Substance Remarks	Xylene – mixture comprised of 15% ortho-, 25% para- and 60% meta-xylene isomers. Supplier not identified
Method	
Method/guideline followed	Guideline not specified
Type	Sister chromatid exchange (SCE) in human peripheral lymphocytes
GLP	Not specified
Year	1993
Species	Human volunteers -5
Strain	Caucasian
Sex	Male
Route of administration	Inhalation
Doses/concentration	40ppm [174mg/m^3],

levels

Exposure period	7hr/day, 3 consecutive days; 3 repeats of exposure at 2wk intervals
Control	5 unexposed male volunteers
Statistical methods	Student's paired t-test (cell cultures from exposed volunteers vs controls). Analysis of variance when needed. P=0.05.
Remarks for Test Conditions.	Five non-smoking white male volunteers [age not specified] with no recent history of occupational exposure to organic solvents were exposed to 40ppm [174mg/m^3] mixed xylene or 40ppm xylene + 50ppm[188.5mg/m^3] toluene for 7hr/day over 3 consecutive days, in an 18.1m^3 dynamic controlled-environment exposure chamber. Solvents were mixed with purified air introduced at a rate of 4.3m^3/min. Chamber concentrations were monitored by gas chromatography and infrared spectrophotometry. The experiment was repeated 3 times at intervals of 2 weeks. Blood samples from each volunteer were taken on each day of the experiment, before and after exposure. Although not specifically described, peripheral blood samples were cultured by the method used for parallel *in vitro* studies. Whole blood lymphocyte cultures were prepared by incubating 0.3ml heparinized whole blood in 5ml RPMI-1640 medium containing fetal calf serum and 1% phytohemaglutinin and were incubated for 72hrs and 37^{0}C. After 48hr, 10µM 5-bromo-deoxyuridine (BrdU) was added to label chromosome strands for SCE, and lymphocytes were harvested 24hr later. Two hours before harvest [46hr in BrdU], 0.1µg/ml colcemid was added to arrest cells division. After 72hr in culture, cells were treated with hypotonic [0.075M] KCl and fixed in absolute methanol/glacial acetic acid [3:1 v/v]. Slides were prepared by the air-drying method, stained by the fluorescene-photolysis-Giemsa method (Perry and Wolff, 1974) and coded for analysis. Slides were analyzed for cell mortality, cell cycle delay, and SCE formation. Forty metaphases in the second round of replication from each preparation were scored for the number of SCE. Cell cycle delay was evaluated in 100cells from each donor by measuring the percentage of mitoses in the first and second [and third] rounds of replication.
Results **Genotoxic effects** **NOAEL (NOEL)** **LOAEL (LOEL)**	NOEL $_{[SCE]}$ = 40ppm [only dose] Significantly increased mortality of cells in culture from individuals exposed to mixed xylene after 3 consecutive days of exposure, 7hr/d, repeated 3 times at 2wk intervals, was observed compared to cell cultures from unexposed controls. Percentage of cell death was approximately 28% (estimated from figure) in cultures from exposed individuals compared to 17% in controls. Mixed exposure

of xylene +toluene did not result in potentiation or additive effects on cell mortality. Effects on cell mortality were no longer seen in cultures 15 hours after cessation of exposure. No significant effects were seen in cells from any individual tested on the rate of sister chromatid exchange and normal cell cycle kinetics [data not shown].

Conclusions (study authors)

Short-term exposure of human volunteers to low level [40ppm] mixed xylene under controlled conditions did not produce significant effects on the cytogenetic parameters, sister chromatid exchange or cell cycle delay in peripheral blood lymphocytes. Increased cell death was observed in cell cultures immediately after exposure but was no longer present 15 hours after exposure was terminated. This pattern of effect was confirmed in three repeat exposure periods at 2-week intervals. Xylene was not a mutagen in humans under conditions of controlled, short-term, low dose exposure.

Data Quality Reliabilities

2. Reliable with restrictions. Actual numerical data was not presented. Cell toxicity values were estimated from histograms. No SCE or cell cycle kinetics data were shown.

References

Richer C.-L., Chakrabarti, S., Senécal-Quevillon, M., Duhr, M.A., Zhang, X.X., and Tardiff, R. 1993. Cytogenetic effects of low-level exposure to toluene, xylene, and their mixture on human blood lymphocytes. Int Arch Occup Environ Health 64: 581-585. [See robust summary on *in vitro* xylene study and *in vivo* study with toluene]
Perry, P., and Wolff, S. 1974. Nature 258: 121-125.

Other
Last changed 2/14/2003

Reproductive Toxicity

Tier 2 - Reproductive Toxicity Robust Summary – API (1983)

Test Substance Remarks

Mixed xylenes (CAS 31330-20-7): 20.4% ortho, 44.2% meta, 20.3% para-xylene, 12.8% ethyl benzene from Appendix Z

Method
Method/guideline followed

Not specified, Standard methods employed

Test type	1-generation reproduction study with teratology segment [high dose and control]
GLP	Not specified
Year	1983
Species/Strain	Rats/ Sprague Dawley from Charles River Breeding Laboratories, St. Constance, Ontario
Route of administration	Inhalation
Duration of test	185 days maximum
Concentration levels	0, 60, 250 and 500ppm distributed as follows: Group II 60ppm 10M, 20F both sexes treated Group III 250ppm 10M, 20F both sexes treated Group IV 500ppm 20M, 40F both sexes treated [½ pregnant females killed at GD 20 for teratology] Group V 500ppm 10M treated, 20F untreated Group VI 500ppm 10M untreated, 20F treated
Sex	Males and females
Exposure period	131 days premating, 20 days mating (M&F); Gestation day (GD) 1-20, Lactation day (LD) 5-20 (F only)
Frequency of treatment	6hr/day, 7 days/week
Control group and treatment	SD rats 30M, 60F chamber-exposed (sham air) [20 pregnant females killed at GD 20 for teratology]
Statistical methods	Statistics performed between data from control and treated groups II-IV, between controls and groups V and VI and for teratology between control and group IV.
Remarks for Test Conditions.	Male and female SD rats, 30 days old, were received from the supplier and acclimated for 20 days. Nine days prior to initiation of treatment, animals were assigned to groups based on body weight. During the study animals were housed individually in stainless steel wire mesh cages during exposure and non-exposure periods except during mating or during lactation (females with litters during non-exposure periods). Animal rooms were maintained at 72-75^{0}F and 50±10% relative humidity with a 12-hour light/dark cycle. Bedding material (hardwood shavings) was provided to pregnant animals on GD 21 through LD 14. Animals were given Purina Rat Chow meal and tap water *ad libitum* except during exposure.

Exposure: Males and females of groups II-IV, group V males and group VI females were treated daily during the 131-day pre-mating period. Males and unmated females continued to receive appropriate exposure during the mating period. Mated females were treated GD 1-20 and LD5-20. Females were removed from their litters during exposure. Mated females were not treated from GD21 to LD 4. Control animals (Group I), Group V females and Group VI males were chambered as sham-air controls for the same 6hr/day, 7 days/wk as treated animals. Unmated/non-pregnant females from the first mating continued to be treated daily through the special mating [see below]. Once mated, they were no longer treated.

Mating: Two females and one male cohabited overnight and each morning vaginal smears were examined for the presence of sperm, which was designated Day 1 of gestation (GD1). Sperm-positive females were removed from the mating cages and housed individually for the duration of gestation. After the first 8 days of mating, males were reassigned to unmated females in the same dose group (1M:1F/mating). Mating period was 21 days.

Special mating: When all litters from regular matings had been delivered, unmated or non-pregnant treated and control females were housed with proven fertility untreated males from the laboratory's in-house breeding colony for up to 15 days. Females identified as sperm-positive were housed individually for gestation but were no longer treated.

Evaluations: Animals were examined twice daily (morning and afternoon) pre- and post-exposure for mortality and toxicologic effects. Physical examinations were performed weekly following exposure. All animals were weighed weekly during pre-mating exposure period, males and unmated females were weighed weekly during mating and post-mating intervals. Mated females were weighed on GD 1, 7, 13, 19 and 21 and LD 1, 7, 14, and 21. Food consumption was recorded during gestation for all mated females at 2-day intervals during GD 1-21. Food was not measured for males or non-pregnant females.

Parturition and Litters: On GD 21 pregnant females were provided with bedding material and were examined twice daily (morning and afternoon) for signs of parturition, which was designated Day 1 of Lactation (LD 1). On LD 14 pans containing bedding material were replaced with wire mesh floors in each cage. Each litter was examined soon after birth on LD 1 for the number of live and dead pups. Litters were observed morning and afternoon and dead pups were removed. Number of pups was recorded on LD4 (prior to cross-fostering and after reassignment), LD 14 and LD 21.

Cross fostering: On LD 4, all pups were removed from their original litter and pooled with other pups of the same age and dose group. Pups were randomly distributed as 4M, 4F/litter to dams on that LD 4. On some days there were not sufficient litters for cross fostering within a dose group. If the available litter had more than 8 pups, it was culled to 8 (4M, 4F if possible); if less than 8 pups, litter remained intact. After LD4, dams nursed the same pups from LD5-21. Pups were not individually identified, pre- or post-fostering, nor identified with litter mates or original dam with the exception of litters culled only or left intact.

Pup Observations: Pups were weighed individually on LD 1, 4, 14, and 21 and given gross external examinations on LD 1, 4 and 21. Pups not selected for complete necropsy on LD21 were weaned and maintained untreated on a basal diet for 28 days post-weaning. Litters remained intact during days 21-28 then pups were separated into male and female group cages for the day 28-49 intervals. Pups were observed morning and afternoon daily for mortality. Dead pups were removed and discarded. No weight data was collected except at sacrifice on day 49 post-partum.

Necropsy: Parental animals found dead or killed moribund were given a gross postmortem examination and discarded with the exception of any abnormal tissue, which was preserved in 10% neutral buffered formalin.
Males: One-half of each group was sacrificed after completion of mating, and the remaining males were sacrificed 21 days later. All males were given gross postmortem examinations. Testes, epididymides, seminal vesicles, prostate and any abnormal tissues were preserved. Testes were weighed.

Females: Teratology: Twenty control and 12 group IV (500ppm) pregnant females were sacrificed on GD 21. A smaller number of pregnant females were selected from the high dose group to assure 20 litters would be available for delivery and subsequent offspring evaluation. Females were given a gross postmortem examination and abnormal tissues were saved. Liver and kidneys were weighed and discarded. Gravid uterus with ovaries attached was weighed and evaluated for number and relative location of implantation sites along both horns for live fetuses, dead fetuses (no visible degeneration), late resorptions (degenerating fetus and placental tissue), and early resorptions (placental remnant). Fetuses were removed and examined for malformations (including palate), weighed and sexed grossly. All resorption sites and placentae were removed and the uterus was reweighed empty. Ovaries were dissected free from the uterus and corpora lutea were counted.

Fetal evaluation: One-half of fetuses from each litter were processed for soft tissue evaluation using the Staples micro-dissection technique. Fetuses were examined under a dissecting microscope and sexes confirmed. Heads were removed and fixed in Bouin's solution for free-hand razor blade sectioning and evaluation of transverse head sections under the dissecting microscope. The decapitated skeletons were stained with Alizarin Red S for skeletal evaluation. The other half of the fetuses from each litter including dead fetuses were processed for skeletal evaluation: ossification variations and skeletal malformations were recorded. At evisceration prior to staining, sexes were confirmed.

Day 21 of Lactation: Females with litters were killed and given a gross postmortem examination. Uterus and ovaries and abnormal tissues were preserved in 10% neutral buffered formalin.

Special Matings: Females that delivered litters were sacrificed on LD 2. Reproductive tracts (ovaries, uterus) and abnormal tissues were saved. Females that mated but did not deliver and females that did not mate were sacrificed on post-mating day 26 or day 22 respectively. Reproductive tracts and any abnormal tissues were saved.

F1 pups Lactation day 21: Randomly selected pups (1/sex/litter) from control and all dose groups were sacrificed, given a gross postmortem examination and 30 tissues including the reproductive organs were preserved in formalin. Testes or ovaries were weighed for each pup, respectively.

F1 pups Post-weaning: On day 49 post-partum, randomly selected pups (1/sex/litter) from control and all dose groups were sacrificed, given a gross postmortem examination and 30 tissues including the reproductive organs were preserved in formalin. Testes or ovaries were weighed for each pup, respectively. Remaining pups were weighed and discarded.

Dead pups: Pups found dead during lactation were given a gross external examination; stomach was evaluated for milk, visceral contents examined grossly. Pups found dead prior to LD 4 were preserved in 70% ethanol; those found dead LD 5-21 were evaluated and discarded.

Results

NOAEL parental 500ppm

NOAEL F1 generation 500ppm

Parental animals: No mortality occurred in any xylene-exposed group. In controls, 1 male died in week 12 and 1 female was sacrificed moribund on week 13. During lactation, two females died on LD 4 and 8. In the parental groups, no treatment related effects were seen in body weight or weight gain during the pre-mating period,

in-life physical observation data, maternal weight data or food consumption/feed efficiency during gestation or in gross postmortem data. Weight data for the reproductive organs of males sacrificed at completion of the mating period showed no treatment related effects and histological evaluations were comparable to controls. Male mating index and fertility index were comparable among exposed and control rats. Although the female mating index was significantly below the 100% in controls, 85% in the 250ppm (both sexes treated), and 85% in the 500ppm (females only treated) groups; a significant decrease in mating did not occur at the 500ppm (both sexes treated – 90%) and 500ppm (males only treated – 95%) groups. In addition, the group VI (females only treated) females mated at a slower rate [10 days] than controls [2 days] or group IV (both sexes treated) at 4 days, a duration similar to controls. The authors did not consider either of these differences to be xylenes-induced because the effects were not seen in the highest dose when both sexes were treated and the controls had an unusually high mating performance. Pregnancy/fertility indices were comparable between controls and each treated group.

Teratology: The mean number of corpora lutea and implantation sites was comparable between control (20 females) and group IV (12 females; both sexes treated). A non-statistically significant increase in mean number of resorption sites and mean % resorptions in relation to implants were seen in group IV animals. Number of pups/litter and sex ratio did not differ between treated and control litters. Mean fetal weights for the high dose group were lower than controls but only the female data were statistically significant. A high incidence of fetuses with at least one ossification variation was observed in both the control and high dose groups, while the high dose had a slightly higher incidence. No increase in soft tissue or skeletal malformations was observed.

Parturition and litters: No adverse effects were evident in gestation length, parturition data, litter size and pup survival data. Average live litter size at birth was 9.6pups in control and 10.8 to 12.5 pups in all treated groups. Number of dead pups at birth was comparable between control and all treated groups. Pup viability index on LD1-4 was 96-99% in all groups and 95-100% in LD4-21 postfostering.

F1 pups: Mean pup weight (sexes combined) in groups II, III, IV in which both parents were treated were statistically significantly lower than control values post-fostering on LD4 but gradually recovered so that by LD21, only group IV (500ppm) was slightly statistically significantly lower than controls [32.9g vs 36.7g]. These mean weights continued to be depressed in group IV offspring at postnatal day 49 sacrifice. Despite these marginal decreases in pup weight in the 500ppm (both sexes treated) group, no decrease in pup body weight was observed in the 500ppm (females only treated) group. Thus, these decreases were not considered adverse effects of treatment by the study authors. Female pups sacrificed at weaning in

the 250 and 500ppm groups had statistically significant decreases in absolute and relative ovary weight at 21 days of age, but the decreases were not concentration-related and were not observed at 49 days of age. Weight of male reproductive organs in pups sacrificed at LD 21 and postnatal day 49 were comparable to controls for all treated groups. Gross postmortem evaluation of LD 21 pups in controls and all treated groups and histological evaluation of tissues from high-dose pups did not reveal any adverse effects of treatment. Special Matings: Females that were unsuccessful breeders in the initial mating: 4, 3, 7, 5, 3, and 4 in controls, 60, 250, 500, 500 (males only treated), and 500ppm (females only treated) were equally unsuccessful when remated with proven breeder males both in the control and treated groups. The maximum successful pregnancy incidence was 2/3 mated females in groups 500 (both sexes treated) and 500 (males only treated). No successful pregnancies occurred in the control group.

Conclusions (contractor)

Exposure to mixed xylenes at concentrations up to 500ppm did not have any toxicologically significant effects on male and female reproductive performance. Litter size, pup survival and sex ratios were unaffected by exposure. Slight effects on female mating index and slight decreases in pup weights were not consistently observed in groups with similar exposure regimens and were therefore not considered adverse effects of treatment. No soft tissue or skeletal malformations were observed in the teratology segment of this study.

Data Quality Reliabilities

1. Reliable without restriction.

References

American Petroleum Institute Medical Report Publication 31-31481. 1983. Parental and fetal reproduction inhalation toxicity study in rats with mixed xylenes. Bio/dynamics, Inc. Project # 80-2520. East Millstone, NJ

Other Last changed

9/15/05

Developmental toxicity

Tier 2: Developmental Toxicity Robust Summary 1a (m-xylene) – Saillenfait (2003)

Test Substance Remarks

Meta-xylene, CAS #108-38-3. =99.5% pure, from Fluka Chemie AG, Buchs, Switzerland

Method Method/guideline followed	EPA OPPTS 870.3700 (1998); OECD protocol 414 (2001)
Test type	Prenatal developmental toxicity
GLP	Not specified
Year	2003
Species	Rat
Strain	Sprague Dawley from CREDO Breeding Laboratories, France
Route of administration	Inhalation
Concentration levels	0, 100, 500, 1000 and 2000ppm
Sex	Pregnant females (22-24/group)
Exposure period	Gestation day (GD) 6-20
Frequency of treatment	6hr/day, 7 days/wk
Control group and treatment	21 pregnant rats, filtered room air
Duration of test	approx. 3-4 weeks (mating interval, 21 days of gestation)
Statistical methods	One-way analysis of variance, Dunnett's test for corpora lutea, implantation sites and live fetuses, maternal food consumption and various body wts; Kruskal-Wallis test, Mann-Whitney test for % of non-live implants, males, proportion of fetuses with alterations per litter; Fisher's test; least squares analysis where applicable for rates of pregnancy, % litters with malformations or variations. Level of significance, $p<0.05$. Litter was basis for analysis of fetal variables.
Remarks for Test Conditions.	Nulliparous female rats (180-200g at mating) had been acclimated for 2 weeks prior to being housed overnight with adult males of same strain and supplier (1M:2F). Day 0 of gestation was the day on which vaginal smears were sperm-positive. Mated females were assigned to treatment groups (23-26 bred rats/group) using a randomization system stratified by body wt on GD0, and housed singly in polycarbonate cages with stainless steel wire lids and corn cob granular bedding, in a room maintained at 21±2^{0}C, 50±5% relative humidity and a 12hr light/dark cycle. Food pellets and

filtered tap water were available ad lib except during exposure. For exposure, rats were transferred to stainless steel wire mesh cages that were placed in the exposure chambers; after exposure animals were returned to original cages and room. Bred females were exposed to 0 (filtered room air), 100, 500, 1000 or 2000ppm m-xylene, 6hr/day, 7 days/wk from GD6-20 in 200 liter glass and steel inhalation chambers with laminar air flow (6-8m^3/hr) at negative pressure of =3mm water, 23±2^{0}C, 50±5% relative humidity. Meta-xylene was delivered by passing additional air through the fritted disk of a heated bubbler; specific chamber concentrations were obtained by varying the temperature of the bubbler and/or air flow rate through the fritted disk. Chamber samples were collected on activated charcoal, desorbed with carbon disulfide, and analyzed on a Shimadzu GC-8A gas chromatograph with flame ionization detector. Internal calibration using toluene was performed.
Food consumption was measured on GD6-13 and GD13-21. Maternal body wt was recorded on GD0, 6, 13, and 21 and weight changes calculated from GD0-6, 6-13 and 13-21. Corrected wt gain was the body wt gain between GD6-21 subtracted from the gravid uterus wt. Females were killed on GD21, the uterus was removed and weighed. Number of corpora lutea, implantation sites, resorptions and dead and live fetuses were recorded. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to ascertain pregnancy and identify any very early resorptions. Live fetuses were weighed, sexed and examined for external anomalies. One-half of live fetuses/litter were fixed in Bouin's solution and examined for soft tissue changes; one-half were fixed in 70% ethanol, eviscerated and processed for skeletal examination and stained with Alizarin Red.

Results

NOAEL maternal toxicity

500ppm

NOAEL developmental toxicity

500ppm
Analytical concentrations were 100±3, 503±10, 1002±23, and 1989±59ppm

Maternal effects

All dams survived exposure. Maternal body wt was decreased significantly at GD13 and GD21 in 2000ppm group. Weight gain and food consumption were reduced for the entire exposure period for 2000ppm dams and during the first half [GD6-13] at 1000ppm. Corrected wt gain was depressed at 1000 and 2000ppm.

Embryo/fetal effects

No significant effects were observed in the average number of implants and live fetuses, the incidence of non-live implants and resorptions or in fetal sex ratios. Fetal body wt (male, female and combined sexes) were significantly lower than controls at 1000 (6%)

and 2000 (14-15%) ppm. Malformations and variations occurred randomly and at comparable incidence across all treated groups and controls, except for a slight statistically significant increase in the incidence of incompletely ossified thoracic centra in fetuses in the 2000ppm group; increased individual incidence did not affect the number of litters with skeletal variations or %fetuses with variations/litter parameters.

Conclusions (study authors)	Maternal toxicity from exposure to m-xylene, expressed variously as statistically significant decrease in body wt, body wt gain and reduced food consumption, occurred at 1000 and 2000ppm. Decreased fetal body wt was observed in litters of the 1000 and 2000ppm groups, concentrations that also caused maternal toxicity. Retarded ossification of thoracic centra occurred only in fetuses at 2000ppm. No teratogenic effects were observed at any dose level.
Data Quality Reliabilities	**1.** Reliable without restrictions
Reference	Saillenfait, A.M., Gallissot, F., Morel, G., and Bonnet, P. 2003. Developmental toxicities of ethylbenzene, *ortho-, meta-, para*-xylene and technical xylene in rats following inhalation exposure. Fd Chem Toxicol 41: 415-429.
Other Last changed	1/17/2003 Revised 9-02-05

Tier 2: Developmental Toxicity Robust Summary 1b (o-xylene) – Saillenfait (2003)

Test Substance Remarks	Ortho-xylene, CAS #95-47-6. =99.5% pure, from Fluka Chemie AG, Buchs, Switzerland
Method Method/guideline followed	EPA OPPTS 870.3700 (1998); OECD protocol 414 (2001)
Test type	Prenatal developmental toxicity
GLP	Not specified
Year	2003
Species	Rat

Strain	Sprague Dawley from CREDO Breeding Laboratories, France
Route of administration	Inhalation
Concentration levels	0, 100, 500, 1000 and 2000ppm
Sex	Pregnant females (20-24/group)
Exposure period	Gestation day (GD) 6-20
Frequency of treatment	6hr/day, 7 days/wk
Control group and treatment	21 pregnant rats, filtered room air
Duration of test	approx. 3-4 weeks (mating interval, 21 days of gestation)
Statistical methods	One-way analysis of variance, Dunnett's test for corpora lutea, implantation sites and live fetuses, maternal food consumption and various body wts; Kruskal-Wallis test, Mann-Whitney test for % of non-live implants, males, proportion of fetuses with alterations per litter; Fisher's test; least squares analysis where applicable for rates of pregnancy, % litters with malformations or variations. Level of significance, $p<0.05$. Litter was basis for analysis of fetal variables.
Remarks for Test Conditions.	Nulliparous female rats (180-200g at mating) had been acclimated for 2 weeks prior to being housed overnight with adult males of same strain and supplier (1M:2F). Day 0 of gestation was the day on which vaginal smears were sperm-positive. Mated females were assigned to treatment groups (23-26 bred rats/group) using a randomization system stratified by body wt on GD0, and housed singly in polycarbonate cages with stainless steel wire lids and corn cob granular bedding, in a room maintained at 21 ± 2^0C, 50±5% relative humidity and a 12hr light/dark cycle. Food pellets and filtered tap water were available ad lib except during exposure. For exposure, rats were transferred to stainless steel wire mesh cages that were placed in the exposure chambers; after exposure animals were returned to original cages and room. Bred females were exposed to 0 (filtered room air), 100, 500, 1000 or 2000ppm o-xylene, 6hr/day, 7 days/wk from GD6-20 in 200 liter glass and steel inhalation chambers with laminar air flow (6-8m^3/hr) at negative pressure of =3mm water, 23 ± 2^0C, 50±5% relative humidity. Ortho-xylene was delivered by passing additional air through the fritted disk of a heated bubbler; specific chamber concentrations were obtained by varying the temperature of the bubbler and/or air flow rate through the fritted disk. Chamber samples were collected on activated charcoal, desorbed with carbon disulfide, and analyzed on

a Shimadzu GC-8A gas chromatograph with flame ionization detector. Internal calibration using toluene was performed. Food consumption was measured on GD6-13 and GD13-21. Maternal body wt was recorded on GD0, 6, 13, and 21 and weight changes calculated from GD0-6, 6-13 and 13-21. Corrected wt gain was the body wt gain between GD6-21 subtracted from the gravid uterus wt. Females were killed on GD21, the uterus was removed and weighed. Number of corpora lutea, implantation sites, resorptions and dead and live fetuses were recorded. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to ascertain pregnancy and identify any very early resorptions. Live fetuses were weighed, sexed and examined for external anomalies. One-half of live fetuses/litter were fixed in Bouin's solution and examined for soft tissue changes; one-half were fixed in 70% ethanol, eviscerated and processed for skeletal examination and stained with Alizarin Red.

Results

NOAEL maternal toxicity

500ppm

NOAEL developmental toxicity

100ppm
Analytical concentrations were 100±4, 499±6, 1017±39, and 1986±55ppm

Maternal effects

All dams survived exposure. Maternal body wt was decreased significantly at GD13 and GD21 in 2000ppm group and at GD21 at 1000ppm. Weight gain was significantly decreased for the entire exposure period and corrected wt gain was decreased for 1000 and 2000ppm dams. Food consumption was reduced at 2000ppm throughout exposure and during GD6-13 [also affecting GD6-21] at 1000ppm.

Embryo/fetal effects

No significant effects were observed in the average number of implants and live fetuses, the incidence of non-live implants and resorptions or in fetal sex ratios. Concentration- related reduction in fetal body wt (male, female, combined sexes) occurred at 500ppm (5%) and above. No statistically significant increases were observed in malformation and variations, with the exception of increased incidence of all types of skeletal variations for the total number of fetuses and fetuses/litter at 2000ppm. Distended ureters were observed a few fetuses from 1, 5, 4, 4, and 0 litters in 0 (control), 100, 500, 1000 and 2000ppm groups, respectively. Diaphragmatic hernia was observed in I fetus from 1 litter each in the 500 and 1000ppm groups.

Conclusions (study authors)

Maternal toxicity from exposure to o-xylene, expressed as statistically significant decrease in body wt, body wt gain and

	reduced food consumption, occurred at 1000 and 2000ppm. Decreased fetal body wt was observed in litters of the 500ppm group in absence of maternal toxicity and at 1000 and 2000ppm groups. Skeletal variations of all types were increased at 2000ppm but no single variant or group of variants were predominant. No teratogenic effects were observed at any dose level.
Data Quality Reliabilities	**1.** Reliable without restrictions
Reference	Saillenfait, A.M., Gallissot, F., Morel, G., and Bonnet, P. 2003. Developmental toxicities of ethylbenzene, *ortho-, meta-, para-*xylene and technical xylene in rats following inhalation exposure. Fd Chem Toxicol 41: 415-429.
Other	
Last changed	1/17/2003, Revised 9-02-05

Tier 2: Developmental Toxicity Robust Summary 1c (p-xylene) – Saillenfait (2003)

Test Substance Remarks	Para-xylene, CAS #106-42-3. =99.5% pure, from Fluka Chemie AG, Buchs, Switzerland
Method	
Method/guideline followed	EPA OPPTS 870.3700 (1998); OECD protocol 414 (2001)
Test type	Prenatal developmental toxicity
GLP	Not specified
Year	2003
Species	Rat
Strain	Sprague Dawley from CREDO Breeding Laboratories, France
Route of administration	Inhalation
Concentration levels	0, 100, 500, 1000 and 2000ppm
Sex	Pregnant females (22-26/group)
Exposure period	Gestation day (GD) 6-20

Frequency of treatment 6hr/day, 7 days/wk

Control group and treatment 25 pregnant rats, filtered room air

Duration of test approx. 3-4 weeks (mating interval, 21 days of gestation)

Statistical methods One-way analysis of variance, Dunnett's test for corpora lutea, implantation sites and live fetuses, maternal food consumption and various body wts; Kruskal-Wallis test, Mann-Whitney test for % of non-live implants, males, proportion of fetuses with alterations per litter; Fisher's test; least squares analysis where applicable for rates of pregnancy, % litters with malformations or variations. Level of significance, $p<0.05$. Litter was basis for analysis of fetal variables.

Remarks for Test Conditions. Nulliparous female rats (180-200g at mating) had been acclimated for 2 weeks prior to being housed overnight with adult males of same strain and supplier (1M:2F). Day 0 of gestation was the day on which vaginal smears were sperm-positive. Mated females were assigned to treatment groups (23-26 bred rats/group) using a randomization system stratified by body wt on GD0, and housed singly in polycarbonate cages with stainless steel wire lids and corn cob granular bedding, in a room maintained at 21±2^{0}C, 50±5% relative humidity and a 12hr light/dark cycle. Food pellets and filtered tap water were available ad lib except during exposure. For exposure, rats were transferred to stainless steel wire mesh cages that were placed in the exposure chambers; after exposure animals were returned to original cages and room. Bred females were exposed to 0 (filtered room air), 100, 500, 1000 or 2000ppm p-xylene, 6hr/day, 7 days/wk from GD6-20 in 200 liter glass and steel inhalation chambers with laminar air flow (6-8m^3/hr) at negative pressure of =3mm water, 23±2^{0}C, 50±5% relative humidity. Para-xylene was delivered by passing additional air through the fritted disk of a heated bubbler; specific chamber concentrations were obtained by varying the temperature of the bubbler and/or air flow rate through the fritted disk. Chamber samples were collected on activated charcoal, desorbed with carbon disulfide, and analyzed on a Shimadzu GC-8A gas chromatograph with flame ionization detector. Internal calibration using toluene was performed.
Food consumption was measured on GD6-13 and GD13-21. Maternal body wt was recorded on GD0, 6, 13, and 21 and weight changes calculated from GD0-6, 6-13 and 13-21. Corrected wt gain was the body wt gain between GD6-21 subtracted from the gravid uterus wt. Females were killed on GD21, the uterus was removed and weighed. Number of corpora lutea, implantation sites, resorptions and dead and live fetuses were recorded. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to ascertain pregnancy and identify any very early resorptions. Live

fetuses were weighed, sexed and examined for external anomalies. One-half of live fetuses/litter were fixed in Bouin's solution and examined for soft tissue changes; one-half were fixed in 70% ethanol, eviscerated and processed for skeletal examination and stained with Alizarin Red.

Results	
NOAEL maternal toxicity	500ppm
NOAEL developmental toxicity	500ppm Analytical concentrations were 100±4, 499±15, 1018±47, and 2001±47ppm
Maternal effects	All dams survived exposure. Maternal body wt was decreased significantly at GD13 and GD21 in 2000ppm group. Weight gain was significantly decreased for the entire exposure period and corrected wt gain was decreased for 1000 and 2000ppm dams. Food consumption was reduced at 2000ppm throughout exposure and during GD6-13 [also affecting GD6-21] at 1000ppm.
Embryo/fetal effects	No significant effects were observed in the average number of implants and live fetuses, the incidence of non-live implants and resorptions or in fetal sex ratios. Fetal body wt (male, female and combined sexes) were significantly decreased at 1000[5-6%] and 2000 [16%] ppm. The only malformation observed was a diaphragmatic hernia in 1 fetus/1 litter at 2000ppm. Incidence of external and visceral variations was low and scattered. The overall incidence of skeletal variations and % fetuses/litter showing skeletal variations were significantly higher than controls in the 2000ppm group; this increase was not attributable to any specific skeletal variation.
Conclusions (study authors)	Maternal toxicity from exposure to p-xylene was expressed as statistically significant decrease in body wt at 2000ppm, and decreased body wt gain and reduced food consumption at 1000 and 2000ppm. Decreased fetal body wt was observed only in litters of the 1000 and 2000ppm groups, concentrations that also caused maternal toxicity. Incidence of skeletal variations of all types was increased at 2000ppm but no single variant or group of variants were predominant. No teratogenic effects were observed at any dose level.
Data Quality Reliabilities	**1.** Reliable without restrictions
Reference	Saillenfait, A.M., Gallissot, F., Morel, G., and Bonnet, P. 2003. Developmental toxicities of ethylbenzene, *ortho-, meta-, para*-xylene

	and technical xylene in rats following inhalation exposure. Fd Chem Toxicol 41: 415-429.
Other **Last changed**	1/17/2003 Revised 9/02/05

Tier 2: Developmental Toxicity Robust Summary 1d (mixed xylenes) – Saillenfait (2003)

Test Substance Remarks	Technical xylene, CAS #1330-20-7. 15.3% ethylbenzene, 21.3%, ortho-, 43.9% meta-, and 19.4% para-xylene, from Fluka Chemie AG, Buchs, Switzerland
Method	
Method/guideline followed	EPA OPPTS 870.3700 (1998); OECD protocol 414 (2001)
Test type	Prenatal developmental toxicity
GLP	Not specified
Year	2003
Species	Rat
Strain	Sprague Dawley from CREDO Breeding Laboratories, France
Route of administration	Inhalation
Concentration levels	0, 100, 500, 1000 and 2000ppm
Sex	Pregnant females (20-24/group)
Exposure period	Gestation day (GD) 6-20
Frequency of treatment	6hr/day, 7 days/wk
Control group and treatment	24 pregnant rats, filtered room air
Duration of test	approx. 3-4 weeks (mating interval, 21 days of gestation)
Statistical methods	One-way analysis of variance, Dunnett's test for corpora lutea, implantation sites and live fetuses, maternal food consumption and various body wts; Kruskal-Wallis test, Mann-Whitney test for % of non-live implants, males, proportion of fetuses with alterations per litter; Fisher's test; least squares analysis where applicable for rates

of pregnancy, % litters with malformations or variations. Level of significance, $p<0.05$. Litter was basis for analysis of fetal variables.

Remarks for Test Conditions.

Nulliparous female rats (180-200g at mating) had been acclimated for 2 weeks prior to being housed overnight with adult males of same strain and supplier (1M: 2F). Day 0 of gestation was the day on which vaginal smears were sperm-positive. Mated females were assigned to treatment groups (23-26 bred rats/group) using a randomization system stratified by body wt on GD0, and housed singly in polycarbonate cages with stainless steel wire lids and corn cob granular bedding, in a room maintained at 21 ± 2^{0}C, 50±5% relative humidity and a 12hr light/dark cycle. Food pellets and filtered tap water were available ad lib except during exposure. For exposure, rats were transferred to stainless steel wire mesh cages that were placed in the exposure chambers; after exposure animals were returned to original cages and room. Bred females were exposed to 0 (filtered room air), 100, 500, 1000 or 2000ppm technical xylene, 6hr/day, 7 days/wk from GD6-20 in 200 liter glass and steel inhalation chambers with laminar air flow (6-8m^3/hr) at negative pressure of =3mm water, 23 ± 2^{0}C, 50±5% relative humidity. Technical xylene was delivered at a constant rate either with an HPLC pump for 2000ppm or with infusion pumps for other concentrations. Liquid chemical was delivered at the top of a heated glass column filled with glass beads and heated compressed air was introduced in the bottom of the glass column countercurrent to liquid flow. Chamber samples were collected on activated charcoal, desorbed with carbon disulfide, and analyzed on a Shimadzu GC-8A gas chromatograph with flame ionization detector. Internal calibration using toluene was performed. Concentrations of technical xylene were obtained by adding the analytical concentrations of ethylbenzene, o-, m-, and p-xylenes.
Food consumption was measured on GD6-13 and GD13-21. Maternal body wt was recorded on GD0, 6, 13, and 21 and weight changes calculated from GD0-6, 6-13 and 13-21. Corrected wt gain was the body wt gain between GD6-21 subtracted from the gravid uterus wt. Females were killed on GD21, the uterus was removed and weighed. Number of corpora lutea, implantation sites, resorptions and dead and live fetuses were recorded. Uteri with no visible implantation sites were stained with 10% ammonium sulfide to ascertain pregnancy and identify any very early resorptions. Live fetuses were weighed, sexed and examined for external anomalies. One-half of live fetuses/litter were fixed in Bouin's solution and examined for soft tissue changes; one-half were fixed in 70% ethanol, eviscerated and processed for skeletal examination and stained with Alizarin Red.

Results

NOAEL maternal toxicity

500ppm

NOAEL developmental toxicity	100ppm Analytical concentrations were 99±3, 499±11, 998±19, and 2004±49ppm
Maternal effects	All dams survived exposure. Maternal body wt was decreased significantly at GD13 and GD21, weight gain and food consumption were significantly decreased for the entire exposure period and corrected wt gain was decreased in 2000ppm dams. Exposure to 1000ppm caused a significant decrease in maternal wt gain between GD6-13 and a non-significant decrease in absolute wt on GD13 and 21; no effect was seen on corrected wt gain or food consumption.
Embryo/fetal effects	No significant effects were observed in the average number of implants and live fetuses, the incidence of non-live implants and resorptions or in fetal sex ratios. Fetal body wt (male, female, combined sexes) were depressed in a concentration-related manner and were significantly lower than controls at 500ppm [4%] and above [maximum decrease 15-17% at 2000ppm]. Visceral malformations occurred sporadically and were evenly distributed in 1-2 fetuses across groups. Occurrences of external, visceral and skeletal variations were comparable to controls for all groups.
Conclusions (study authors)	Maternal toxicity from exposure to technical xylene was expressed as statistically significant decrease in body wt, decreased body wt gain and reduced food consumption at 2000ppm, and as decreased body wt gain at GD6-13 in 1000ppm dams. Concentration related statistically significant decreases in fetal body wt were observed at 500ppm in the absence of maternal toxicity, and also at 1000 and 2000ppm. No significant effects on the incidence of visceral malformations, external, visceral and skeletal variations were observed at any technical xylene-exposed dose level compared to controls. No teratogenic effects were observed at any dose level.
Data Quality Reliabilities	**1.** Reliable without restrictions
Reference	Saillenfait, A.M., Gallissot, F., Morel, G., and Bonnet, P. 2003. Developmental toxicities of ethylbenzene, *ortho-, meta-, para*-xylene and technical xylene in rats following inhalation exposure. Fd Chem Toxicol 41: 415-429.
Other	
Last changed	1/17/2003; Revised 9/02/05

Tier 2: Immunotoxicity Robust Summary 1a (m- xylene) – Condie (1988)

Test Substance	Meta-xylene, CAS #108-38-3, 99% pure (Fisher Scientific)
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Repeat Dose
GLP	Not specified
Year	1988
Species/Strain	Rats Sprague-Dawley
Route of administration	Oral, gavage
Duration of test	10 days
Doses/concentration levels	Naïve, 0 (corn oil), 250, 1000, 2000 mg/kg (in corn oil)
Sex	Male and female (10/sex/group)
Frequency of treatment	once daily, 10 consecutive days
Control group and treatment	10M, 10F; corn oil [10ml/kg]
Post exposure observation period	None
Statistical methods	Analysis of variance for homogeneity; Dunnett's t-test; non-homogeneous data subject to Wilcoxin Rank Sum Test
Test Conditions	Male and female rats were received at 5 weeks of age, quarantined for 1 week and assigned to treatment groups by computer randomization. Rats were housed in a room with controlled temp [21-24^{0}C] and humidity [30-70% with 12hr light/dark cycle. Food and water were available *ad libitum.* Five groups (10M, 10F/group) were treated with 0 (naive, not dosed), 0 (corn oil, 10ml/kg), 250, 100 200mg/kg/day m-xylene in corn oil daily for 10 consecutive days. Animals were observed twice daily at 5 hr intervals, body wt determined on day 1, 5 and termination of study [24hr after last dose]. At sacrifice, animals were anesthezized with ether, and blood collected by cardiac puncture for

	hematology and serum chemistry. Urinalysis was performed individually with Labstik reagent strips. Gross pathology was performed and selected organ weighed. Tissues were preserved in 10% buffered formalin but no histopathology was performed
Results	
NOAEL	1000 mg/kg
LOAEL	2000mg/kg [based on spleen wt changes]
	No treatment related deaths observed; no adverse effects on hematology, serum chemistry, or urinalysis. Significantly lower body wt seen in high dose males, no effects on female body wt. <u>Absolute and relative spleen wts were statistically significantly decreased in males at 2000mg/kg with dose related trend at lower doses.</u> Data on female spleens not provided. Absolute and relative liver wts were increased in both sexes at 2000mg/kg
Conclusion	Exposure of rats for 10 days to 2000mg/kg meta-xylene resulted in increased liver wt in both sexes and signficantly decreased spleen weights in males with a dose-related trend at lower doses, which may indicate possible effects on the immune system.
Data Quality	**2.** Reliable with restriction. Incomplete weight data for organs of interest.
Reference	Condie, L.W., Hill, J.R., and Borzellica, J.F. 1988. Oral toxicology studies with xylene isomers and mixed xylene. *Drug Chem Toxicol* 11: 329-354.

Tier 2: Immunotoxicity Robust Summary 1b (o- xylene) – Condie (1988)

Test Substance	Ortho-xylene, CAS #95-47-6, 99% pure (Fisher Scientific)
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Repeat Dose
GLP	Not specified
Year	1988
Species/Strain	Rats Sprague-Dawley

Route of administration	Oral, gavage
Duration of test	10 days
Doses/concentration levels	Naïve, 0 (corn oil), 250, 1000, 2000 mg/kg (in corn oil)
Sex	Male and female (10/sex/group)
Exposure period	13 weeks
Frequency of treatment	once daily, 10 consecutive days
Control group and treatment	10M, 10F; corn oil [10ml/kg]
Post exposure observation period	None
Statistical methods	Analysis of variance for homogeneity; Dunnett's t-test; non-homogeneous data subject to Wilcoxin Rank Sum Test
Test Conditions	Male and female rats were received at 5 weeks of age, quarantined for 1 week and assigned to treatment groups by computer randomization. Rats were housed in a room with controlled temp [21-24^{0}C] and humidity [30-70% with 12hr light/dark cycle. Food and water were available *ad libitum.* Five groups (10M, 10F/group) were treated with 0 (naive, not dosed), 0 (corn oil, 10ml/kg), 250, 100 200mg/kg/day ortho-xylene in corn oil daily for 10 consecutive days. Animals were observed twice daily at 5 hr intervals, body wt determined on day 1, 5 and termination of study [24hr after last dose]. At sacrifice, animals were anesthesized with ether, and blood collected by cardiac puncture for hematology and serum chemistry. Urinalysis was performed individually with Labstik reagent strips. Gross pathology was performed and selected organ weighed. Tissues were preserved in 10% buffered formalin but no histopathology was performed

Results	
NOAEL	1000 mg/kg
LOAEL	2000 mg/kg [based on spleen wt changes] No treatment related deaths observed; no adverse effects on hematology, serum chemistry, or urinalysis. Significantly lower body wt seen in high dose males, no effects on female body wt. Absolute spleen wts were statistically significantly decreased in males at 2000mg/kg with dose related trend for absloute and relative wts at lower doses. Data on female spleens not provided. Absolute and relative liver wts [females] and relative liver wts [males] were increased in both sexes at 2000mg/kg.
Conclusions	Exposure of rats for 10 days to 2000mg/kg ortho-xylene resulted in increased liver wt in both sexes and significantly decreased spleen weights in males with a dose-related trend at lower doses, which may indicate possible effects on the immune system.
Data Quality	**2.** Reliable with restriction. Incomplete weight data for organs of interest.
Reference	Condie, L.W., Hill, J.R., and Borzellica, J.F. 1988. Oral toxicology studies with xylene isomers and mixed xylene. *Drug Chem Toxicol* 11: 329-354.

Tier 2: Immunotoxicity Robust Summary 1c (p- xylene) – Condie (1988)

Test Substance	Para-xylene, CAS #106-42-3, 99% pure (Fisher Scientific)
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Repeat Dose
GLP	Not specified
Year	1988
Species/Strain	Rats Sprague-Dawley
Route of administration	Oral, gavage
Duration of test	10 days

Doses/concentration levels	Naïve, 0 (corn oil), 250, 1000, 2000 mg/kg (in corn oil)
Sex	Male and female (10/sex/group)
Exposure period	13 weeks
Frequency of treatment	once daily, 10 consecutive days
Control group and treatment	10M, 10F; corn oil [10ml/kg]
Post exposure observation period	None
Statistical methods	Analysis of variance for homogeneity; Dunnett's t-test; non-homogeneous data subject to Wilcoxin Rank Sum Test
Test Conditions	Male and female rats were received at 5 weeks of age, quarantined for 1 week and assigned to treatment groups by computer randomization. Rats were housed in a room with controlled temp [21-24^{0}C] and humidity [30-70% with 12hr light/dark cycle. Food and water were available *ad libitum.* Five groups (10M, 10F/group) were treated with 0 (naive, not dosed), 0 (corn oil, 10ml/kg), 250, 100 200mg/kg/day para-xylene in corn oil daily for 10 consecutive days. Animals were observed twice daily at 5 hr intervals, body wt determined on day 1, 5 and termination of study [24hr after last dose]. At sacrifice, animals were anesthesized with ether, and blood collected by cardiac puncture for hematology and serum chemistry. Urinalysis was performed individually with Labstik reagent strips. Gross pathology was performed and selected organ weighed. Tissues were preserved in 10% buffered formalin but no histopathology was performed

Results	
NOAEL	1000 mg/kg
LOAEL	2000 mg/kg [based on thymus wt changes] Two deaths in females given 2000mg/kg occurred; no adverse effects on hematology, serum chemistry, or urinalysis were observed. Significantly lower body wt seen in high dose males, no effects on female body wt. Absolute and relative thymus wts [males] and relative thymus wt [females] were statistically significantly decreased at 2000mg/kg with dose related trend at lower doses. Data on spleen wts was not provided. Absolute and relative liver wts [females] and relative liver wts [males] were increased in both sexes at 2000mg/kg.
Conclusions	Exposure of rats for 10 days to 2000mg/kg para-xylene resulted in increased liver wt in both sexes and significantly decreased thymus weights with a dose-related trend at lower doses, which may indicate possible effects on the immune system.
Reliability	**2.** Reliable with restriction. Incomplete weight data for organs of interest.
Reference	Condie, L.W., Hill, J.R., and Borzellica, J.F. 1988. Oral toxicology studies with xylene isomers and mixed xylene. *Drug Chem Toxicol* 11: 329-354.

Tier 2: Immunotoxicity Robust Summary 1d (mixed xylenes) – Condie (1988)

Test Substance	mixed xylenes, technical grade (in corn oil) o-xylene 17.6%, m- and p-xylene 62.3%, ethylbenzene 20.0%
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Repeat Dose
GLP	Not specified
Year	1988
Species/Strain	Rats Sprague-Dawley
Route of administration	Oral, gavage
Duration of test	90 days

Doses/concentration levels	0 (corn oil), 150, 750, 1500 (in corn oil)
Sex	Male and female (10/sex/group)
Frequency of treatm.	90 consecutive days
Control group and treatment	10M, 10F; corn oil [5ml/kg]
Post exposure observation period	None
Statistical methods	Analysis of variance for homogeneity; Student's t-test
Test Conditions	Male and female SD rats were received at 6.5 weeks of age, quarantined for 1 week and assigned to treatment groups by computer randomization. Rats were housed in a room with controlled temp [21-24^{0}C] and humidity [30-70% with 12hr light/dark cycle. Food and water were available *ad libitum.* Four groups (10M, 10F/group) were treated with 0 (corn oil, 5ml/kg), 150, 750 and 1500mg/kg technical grade mixed xylene in corn oil daily for 90 consecutive days. Animals were observed daily for clinical signs. Body weight was recorded weekly. Urine samples were collected during the final week of study by placing animals in individual metabolism cages. At the end of 13 weeks, blood samples for hematology and serum chemistry were collected by cardiac puncture prior to necropsy. Gross necropsy were performed on all animals and specific organs weighed [brain, liver, spleen, lungs, thymus, kidneys, heart and gonads]. All major organs were preserved in 10% buffered formalin. Liver and kidney specimens from all dose groups were examined microscopically.
Results	NOEL or LOAEL were not determined based on changes in immune system related organs. No deaths were observed in this study. Statistically significant decreased body weight was observed in males treated with 1500mg/kg; female weights were comparable to controls. No adverse effect on urinalysis was seen. Statistically significant variations were seen in some serum chemistry parameters but were not of sufficient magnitude to be considered biologically significant by the authors. Hematology changes indicated the presence of mild polycythemia and leukocytosis in some dose groups but the clinical health of the animals was unaffected. Liver weights

(absolute and relative) were increased at all doses in male rats and in mid and high dose females without histopathological correlate. Kidney weights in males at mid and high doses and in females at high dose were increased. Spleen and thymus weights [thymus weight data not presented] were not affected in males and only high dose females exhibited increased spleen weight [absolute and relative] at 1500mg/kg. Histologically, male rats exhibited increased incidence of hyaline droplets in a dose related pattern and females had minimal dose-related lesions indicative of early stages of chronic nephropathy. The authors pointed out the control data for females for nephropathy was very low making these observations in treated females difficult to interpret.

Conclusion

Exposure of rats for 90 days to mixed xylenes resulted in increased weights of liver and kidneys. Histopathological evaluation of liver and kidney tissue revealed an increased incidence of minimal chronic renal disease only in female rats and hepatic related changes were seen in either sex. Increased spleen weight was observed in high dose females was observed but no other changes in spleen or thymus, organs associated with the immune system, were reported.

Data Quality

2. Reliable with restriction. Incomplete weight data for organs of interest.

Reference

Condie LW, Hill JR et al. Oral toxicity studies with xylene isomers and mixed xylenes. Drug and Chemical Toxicology. 1988. 11. 329-354

Xylene VCCEP Robust Summaries

Tier 3 Studies

Tier 3: Chronic Toxicity/Carcinogenicity

Tier 3: Chronic Toxicity Robust Summary 1a - National Toxicology Program (NTP) (mice) 1986

Test Substance **Remarks**	Mixed xylenes, CAS #1330-20-7; 60% meta-, 14% para-, (% ortho-xylene, and 17% ethyl benzene. Mol wt. 106.2. from Shell Oil Co, Houston, TX. Purity and composition determined by elemental analysis and infrared, UV/visible, and nuclear magnetic resonance analysis.
Method	
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Chronic – 2 year oral carcinogenesis
GLP	Yes
Year	1986
Species/Strain	Mice B6C3F1
Route of administration	Oral, gavage
Duration of test	103 weeks
Doses/concentration levels	0, 500, and 1000mg/kg in corn oil
Sex	Male and female (50/sex/group)
Exposure period	103 weeks
Frequency of treatment	once daily, 5 days/wk
Control group and treatment	50M, 50F; corn oil (8ml/kg)
Post exposure observation period	None
Statistical methods	Kaplan and Meier (1958) for survival curves. Tumor Incidence analysis by 3 methods: Cox (1972) and Tarone's (1972, 1975) Life Table Analysis for dose related effects on

survival and tumor incidence; Incidental tumor analysis – proportion of tumor-bearing animals compared at 5 intervals, wk0-52, wk53-78, wk79-92, wk 93 103 [wk before terminal kill began (wk 104-105)]. Individual time interval comparisons are combined to obtain single overall result (Haseman, 1984); Unadjusted analyses use survival-adjusted methods with Fisher exact test for pair-wise comparison. Cochran-Armitage linear trend test was used on overall proportion of tumor-bearing animals, with no adjustment for survival differences. NTP historical control data were also compared for tumors appearing to show compound-related effects.

Test Conditions

Male and female mice (6wk old at receipt) from Charles River Breeding Laboratories were observed for 19 days. Complete necropsies were performed on 5 mice/sex to assess health status. Mice were placed on study at 8 wk of age, individually weighed to determine the weight range for each sex, then distributed by weight class into polycarbonate cages with polyester filters, 5 rats/cage. Cages were assigned to test groups according to tables of random numbers. Animals were weighed, identified by toe clip, and given food [Purina Lab Chow- analysis supplied] and water *ad libitum*. Animal rooms were maintained at 22±1^{0}C, 40-60% relative humidity with 15 room air changes per hour, and 12hr light/dark cycle. Mixed xylene in corn oil was administered in doses of 0, 500, and 1000mg/kg/day, 5 days/wk for 103 weeks. Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded once a day for 16 months, then once/month. Body weights by cage were recorded once a week for the first 12wks and once/month for the duration of study. Mean body wt were calculated for each group. Food consumption was not recorded. At the end of 103 weeks, surviving mice were killed and necropsies were performed on all animals including those found dead unless they were excessively autolyzed or cannibalized. Organs were not weighed. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and slides stained with hematoxylin-eosin. Tissues from all dose groups examined histopathologically included gross lesions and tissue masses, mandibular lymph nodes, salivary glands, femur including marrow, thyroid, parathyroids, small intestines, colon, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, liver, gall bladder, prostate/testes, ovaries/uterus, mammary gland, lungs and main stem bronchi, adrenals, urinary bladder, pituitary, and eyes [if grossly abnormal]. At completion of the pathological

	evaluation, all records, slides, blocks and tissue counts were verified. Tumor diagnoses of all target tissues and selected 10% of animals were evaluated independently by a quality assessment laboratory.
Results NOAEL (NOEL) LOAEL (LOEL)	NOAEL both sexes = 1000mg/kg
Remarks	Mean body wt of mixed xylene treated mice were comparable to controls throughout the study. Hyperactivity occurred in all 1000mg/kg mice (both sexes) 5-30min after dosing, and was observed consistently during the study from wk4-103. No significant differences in survival were observed between any treated group and controls of either sex. Vehicle control male mice had lower survival at the end of the study than treated groups, attributed to effects of urinary tract infections early in the study and debilitating effects of dorsal fibrosarcoma later in the study. Group housing may have exacerbated these conditions. No significant non-neoplastic or neoplastic lesions were observed in male or female mice of any dose group. Total number of females with malignant tumors at 1000mg/kg was 16/50 compared to 29/50 female controls and total number of males with malignant tumors at 1000mg/kg was 23/50 compared to 26/50 male controls.
Conclusions (study authors)	There was no evidence of carcinogenicity in male or female B6C3F1 mice from treatment with mixed xylenes at oral doses of 500 and 1000mg/kg/day in this 2-year bioassay
Quality Reliabilities	1. Reliable without restrictions.
References	National Toxicology Program. 1986. Toxicology and carcinogenesis studies of xylene (mixed) in F344/N rats and B6C3F1 mice (gavage studies). Performed at Batelle Columbus, OH. Technical Report #327. National Institutes of Health, US DHHS, Washington, DC
Other Last changed	2/14/2003

Tier 3: Chronic Toxicity Robust Summary 1b - National Toxicology Program (NTP) (rat) 1986

Test Substance Remarks	Mixed xylenes, CAS #1330-20-7; 60% meta-, 14% para-, (% ortho-xylene, and 17% ethyl benzene. Mol wt. 106.2. from Shell Oil Co, Houston, TX. Purity and composition determined by elemental analysis and infrared, UV/visible, and nuclear magnetic resonance analysis.
Method	
Method/guideline followed	No guidelines specified. Standard methods employed
Test type	Chronic – 2 year oral carcinogenesis
GLP	Yes
Year	1986
Species/Strain	Rats F344/N
Route of administration	Oral, gavage
Duration of test	103 weeks
Doses/concentration levels	0, 250, and 500mg/kg in corn oil
Sex	Male and female (50/sex/group)
Exposure period	103 weeks
Frequency of treatment	once daily, 5 days/wk
Control group and treatment	50M, 50F; corn oil (4ml/kg)
Post exposure observation period	None
Statistical method	Kaplan and Meier (1958) for survival curves. Tumor Incidence analysis by 3 methods: Cox (1972) and Tarone's (1972, 1975) Life Table Analysis for dose related effects on survival and tumor incidence; Incidental tumor analysis – proportion of tumor-bearing animals compared at 5 intervals, wk0-52, wk53-78, wk79-92, wk 93 103 [wk before terminal kill began (wk 104-105)]. Individual time interval comparisons are combined to obtain single overall result (Haseman, 1984); Unadjusted analyses use survival-adjusted methods with Fisher exact test for pair-wise comparison. Cochran-Armitage linear trend test was used

on overall proportion of tumor-bearing animals, with no adjustment for survival differences. NTP historical control data were also compared for tumors appearing to show compound-related effects.

Test Conditions

Male and female rats (5wk old at receipt) from Charles River Breeding Laboratories were observed for 19 days. Complete necropsies were performed on 5 rats/sex to assess health status. Rats were placed on study at 7 wk of age, individually weighed to determine the weight range for each sex, then distributed by weight class into polycarbonate cages with polyester filters, 5 rats/cage. Cages were assigned to test groups according to tables of random numbers. Animals were weighed, identified by toe clip, and given food [Purina Lab Chow- analysis supplied] and water ad libitum. Animal rooms were maintained at 22 ± 1^0C, 40-60% relative humidity with 15 room air changes per hour, and 12hr light/dark cycle. Mixed xylene in corn oil was administered in doses of 0, 250, and 500mg/kg/day, 5 days/wk for 103 weeks. Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded once a day for 16 months, then once/month. Body weights by cage were recorded once a week for the first 12wks and once/month for the duration of study. Mean body wt were calculated for each group. Food consumption was not recorded. At the end of 103 weeks, surviving rats were killed and necropsies were performed on all animals including those found dead unless they were excessively autolyzed or cannibalized. Organs were not weighed. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and slides stained with hematoxylin-eosin. Tissues from all dose groups examined histopathologically included gross lesions and tissue masses, mandibular lymph nodes, salivary glands, femur including marrow, thyroid, parathyroids, small intestines, colon, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, liver, prostate/testes, ovaries/uterus, mammary gland, lungs and main stem bronchi, adrenals, urinary bladder, pituitary, and eyes [if grossly abnormal]. At completion of the pathological evaluation, all records, slides, blocks and tissue counts were verified. Tumor diagnoses of all target tissues and selected 10% of animals were evaluated independently by a quality assessment laboratory.

Results
NOAEL (NOEL)

NOAEL males = 250mg/kg; females = 500mg/kg

LOAEL males = 500mg/kg [mortality]

LOAEL (LOEL)

Remarks

Mean body wt of male rats given 500mg/kg/day was 5-8% lower than controls after wk 59; weights of 250mg/kg males and all females were comparable to controls throughout the study. Survival of 500mg/kg males was significantly lower than controls after 103wk. Among high dose males, 30/50 died – 19 non-accidental deaths [including moribund rats], 11 accidentally killed [e.g. dosing errors], compared to 14/50 deaths in vehicle controls – 11 non-accidental deaths, 3 accidental deaths. No other differences in survival were observed in 250mg/kg/day males or 500 and 250mg/kg/day females. The only neoplastic events described were considered related to increased mortality. Overall incidence of interstitial cell tumors in testes were comparable to vehicle controls, however survival-adjusted analyses indicated increased incidence in 500mg/kg rats, primarily due to animals dying between wk 62–92; tumor incidence was comparable during other time intervals. This marginal effect was not considered compound-related. Dose-related decreases in the incidence of mononuclear cell leukemia and pituitary gland adenoma or carcinoma combined was observed in male rats [leukemia – 22/50, 18/50, and 11/50; pituitary

adenoma/carcinomas – 24/49, 22/50, and 12/45 in control, 250 and 500mg/kg groups , respectively], due primarily to decreased survival in 500mg/kg/day males compared to controls. The total number of animals with malignant tumors at 500mg/kg was 16/50 females compared to 12/50 female controls, and 19/50 males compared to 32/50 male controls, probably due to decreased survival in high dose males. Overall, the incidences of non-neoplastic and neoplastic lesions in xylene-dosed rats of either sex were not considered related to administration of mixed xylenes at any dose levels.

Conclusions (study authors)

There was no evidence of carcinogenicity in male or female F344/N rats from treatment with mixed xylenes at oral doses of 250 and 500mg/kg/day in this 2-year bioassay

Quality Reliabilities

1. Reliable without restrictions.

References

National Toxicology Program. 1986. Toxicology and carcinogenesis studies of xylene (mixed) in F344/N rats and B6C3F1 mice (gavage studies). Performed at Batelle Columbus, OH. Technical Report #327. National Institutes of Health, US DHHS, Washington, DC

Other	2/14/2003
Last changed	

Tier 3: Adult Neurotoxicity

Tier 3: Adult Neurotoxicity Robust Summary – Korsak (1994)

Test Substance Remarks	Meta-xylene (CAS #108-38-3); purity not specified, from Reachim and Polish Chemical Reagent Co.
Method	
Method/guideline followed	Not specified; similar to standard procedure
Test type	Subchronic
GLP	Not specified
Year	1994 publication
Species/Strain	Rat / Wistar [Imp:DAK stock outbred] (12/group)
Route of administration	Inhalation
Duration of test	3 months
Doses/concentration levels	0, 50, 100ppm [217, 435mg/m^3]
Sex	Male
Exposure period	6hr/day
Frequency of treatment	5 days/wk
Control group and treatment	24 rats, sham-exposed
Post exposure observation period	None
Statistical methods	Analysis of variance, Dunnett's test, Fisher exact test
Test Conditions	Male rats were housed in plastic cages with wire-mesh covers and maintained under 12hr light/dark cycle. Food and water was available ad libitum in home cages. Body wts of all rats were recorded prior to start of study and weekly during the experiment. Rats were exposed in a dynamic chamber of 1.3m^3 volume; vapors were generated by heating, and concentrations were obtained by diluting with air. Concentrations in chamber were measured every

	30 min by GC-FID. Hematologic parameters, measured at initiation and 1 week before termination, included erythrocyte counts (RBC), hemoglobin, hematocrit, leukocyte counts (WBC) and differentials. Clinical chemistries, conducted on all fasted rats 24hr after the last exposure, were alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, protein, albumin, glucose, and electrolytes. Microsomal monooxygenases and lipid peroxidation were measured in liver homogenate, as was alanine p-hydroxylase. Liver microsomes were prepared by $CaCl_2$ aggregation and protein determined by Lowrey method. CYP450 differences were measured by spectrophotometer at wave lengths between 490 and 450nm [Omura and Sato, 1964]. Lipid peroxidation, triglycerides, hepatic lipids were also measured. Rotarod performance [per Kaplan and Murphy, 1972, 8cm diameter rod rotating at 12rpm, 2 min test interval] was evaluated before the experiment began and at each month during the 3-month exposure. Hot plate behavior was tested at termination of exposure to measure level of analgesia. Plate temp. was 54.5^{0}C, animal was removed after licking foot or after 60 sec.
Results	
NOAEL (NOEL)	50ppm
LOAEL (LOEL)	100ppm (based on rotarod decrement)
Remarks	m-Xylene did not alter final body wt or absolute and relative organ wts (heart, lungs, liver, spleen, kidneys, adrenals, testes). At 50 and 100ppm, hemaglobin and RBC counts were slightly but significantly reduced and WBC counts were elevated at 100ppm; there were no effects on differentials. m-Xylene did not affect concentration of microsomal protein, monooxygenase activities, lipid peroxidation, or clinical chemistry parameters. Increased sensitivity to pain [shortened latency to paw lick in hot plate test] was observed at both dose levels but was not enhanced with increased dose. Rotarod performance decreased in a dose responsive manner at 50 and 100ppm reaching statistical significance at 100ppm; the decrement was not affected by duration of exposure (1, 2, or 3 months). These rotarod results were used by EPA in calculating the 2002 inhalation reference dose for xylenes, (IRIS, 2002).
Conclusions (study authors)	m-Xylene behaved as a neurotoxicant to rats by inducing motor coordination disturbances and increased sensitivity to pain in this assay.
Quality	1, Reliable without restrictions

Reliabilities

References

Korsak, Z., Wisniewska-Knypl, J., and Swiercz, R. 1994. Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int J Occup Med Environ Health 7: 155-166.
IRIS. 2002. Toxicological review of xylenes (CAS #1330-20-7). US EPA, Washington, DC.

Kaplan, M.L. and Murphy, S.D. 1972. Toxicol Appl Pharmacol 22: 259-266.

Omura, T., and Sato, R. 1964. J Biol Chem 239: 2370-2378

Other

Last changed 10/29/2002

Tier 3: Developmental Neurotoxicity Robust Summary – Hass 1995, 1997

Test Substance Remarks Mixed xylenes (CAS #1330-20-7). 19% ortho-, 45% meta-, 20% para-xylene, 15% ethylbenzene. Supplier Bie and Berntsen.

Method

Method/guideline followed Not specified. Standard methods employed

Test type Developmental and postnatal neurotoxicity

GLP Not specified

Year 1995

Species Rat

Strain Mol:Wistar from Møllegårds Breeding Center Ltd

Route of administration Inhalation

Concentration levels 0, 500ppm

Sex Pregnant females, initial number not specified; 15 litters delivered

Exposure period Gestation day [GD] 7-20

Frequency of treatment 6hr/day [9:00am to 3:00pm]

Control group and treatment

Pregnant females initial number not specified; 11 litters delivered; inhalation of clean air

Duration of test

GD0-22; postnatal day [PND] 0-22, up to 4 months

Statistical methods

The litter or 1 pup/litter was the statistical unit. Significance level =0.05. When more than 1 pup/litter was investigated, 2 strategies were available to avoid inflation of sample size: 1) Use 1 score/litter either a litter mean or the score of 1 pup/litter, or 2) Include the litter as an independent, random and nested factor in the ANOVA. The three main effects were dose, sex and housing, depending on the task, with usually a repeated measure or trial in days. Litters were nested within dose and pups were nested within litters. If sex or housing appeared in the model, pups did not appear in the model because each pup was uniquely defined by the sex-housing variable. The model is similar to Nelson et al., 1985.

Dependent Variable = Dose + Litter (dose) + Sex + Dose*Sex + Housing + Dose*Housing + Sex*Housing + Dose*Sex*Housing. Developmental milestones, ontogeny of reflexes and rotarod data were analyzed by Mann-Whitney U Test and Chi Square. SYSTAT PC-version software package was employed.

Remarks for Test Conditions.

Young nulliparous female rats were housed in pairs in plastic cages in an animals room under controlled environmental conditions of 22±2^{0}C, 40-60% relative humidity, 8 air changes/hour, 12hr light/dark cycle at 500 lx. Food and water were provided ad lib. Rats were weighed weekly. Females (180-200g) were mated 1 or 2 per male from 8a.m. to 12p.m. and mating was confirmed by sperm in vaginal smear [GD 0]. Sperm-positive females were allocated alternately in to control or exposure group in order of appearance. Females were placed in wire mesh cages (2 per cage) in 800liter inhalation chambers on the day before exposure was initiated for adaptation.
<u>Exposure</u>: On GD 7-20 females were exposed to technical xylene or clean air 6hr/day, 7 days/week. Chambers had a dynamic airflow of 8-12 air changes/hour, an evenly distributed atmosphere and slight negative pressure. Temperature in the chambers was 22-25^{0}C with 35-60% relative humidity. One group of rats was exposed to 500ppm technical xylene; the other group to clean air. Xylene was evaporated into the air inlet from a glass spiral heated by the circulation of warm water (40^{0}C). Xylene concentration was monitored continuously with an infrared gascell spectrophotometer (MIRANR-1A) calibrated daily with known concentrations of xylene. Mean concentration of xylene in the chamber was 499±13ppm. Air samples from charcoal tubes were regularly analyzed by gas chromatography. Rats were given food and water ad libitum except during exposure and were observed daily after exposure for signs of

toxicity. Body weight and food consumption were recorded on GD0, 6, 10, 15, and 20.
Parturition: After exposure on GD20, females were housed alone in plastic cages with nesting material in an animal room. Onset of birth was examined at 8am, 12pm, 4pm and time of birth was recorded. Animals giving birth after 4pm and before 8am the following morning were considered to have given birth at 12am. The expected day of delivery [GD 22] was designated PND0. After parturition was complete, pups were counted, sexed and examined for anomalies. Maternal and pup weights were recorded. Pups found dead were recorded and examined for macroscopic changes when possible. Sizes of litter were not standardized but litters with less than 6 pups were not included in postnatal evaluations.
Weaning: 2 male and 2 female pups per litter with median body weights were weaned on PND 22: 1M, 1F/litter were standard housed in small cages in same-sex pairs with same prenatal exposure and left undisturbed except for feeding and weighing until 3 months of age when they were tested in the Morris water maze. 1M, 1F/litter were group housed (4-5/cage) same-sex, same prenatal exposure in an enriched environment in large cages with various toys. Enriched-housed pups were tested on rotarod, open field and Morris water maze at 3 months of age.
Necropsy: All other pups, dams, and sperm-positive females who had not given birth were killed and examined macroscopically on PND 28 ± 2. Adult females were inspected for implantation sites. Brain weight of one randomly chosen pup/sex/litter was recorded.
Postnatal Growth, Physical and Reflex Development: Body weights of offspring were recorded preweaning on PND 1, 2, 3, 6, 10, 14, 19, and 22. Physical and reflex development examination of all pups included ear unfolding, surface righting, homing response, incisor eruption, auditory startle, eye opening, air righting. After weaning, selected pups were weighed once every 2 weeks until study termination at approximately 4 months. Sexual maturation was determined by vaginal opening in females and cleavage of balano-preputial skinfold in males.
Behavioral Testing: All testing was performed during the animals' dark period (i.e. active period). Investigator did not know which exposure group the animals were from but did know housing group.
a) Motor activity (**Rotarod**): 1M, 1F/litter/group from the enriched housed animals were tested on PND 24, 25, and 26 at speeds of 10, 12, or 15rpms, respectively for 30 seconds. Rats were scored for time on the rod and % of animals in each group reaching 30 seconds.
b) Activity (**Open Field**): 1M, 1F/litter/group from the enriched housed animals were tested for 3 minutes in a square open field (75 cm) on PND 27 and 34 ± 2 using contrast sensitive computer controlled video tracking. Total distance moved in field, % time spent in the center square of the field were calculated and the

number of defecations counted.
c) Learning and Memory (**Morris Water Maze**): Offspring from standard housed and enriched housed groups were tested at 3 months of age in a black plastic pool filled to a depth of 30cm with water at 22^{0}C and equipped with a circular platform (diameter 10 cm) on a solid block situated 1cm below the water surface. Four arbitrary points on the rim of the pool designated as N, S, E, and W served as starting points and divided the pool into quadrants. Animals were tested in blocks of 4 trials using the 4 starting points in a pseudo-random sequence. Two standard cages (2 rats/cage) and one large enriched housing cage (4-5 rats/cage) were placed near the pool and one animal after another was started from the first point, tested and returned to the cage. The procedure was repeated at each starting point. When a rat swam to and climbed onto the platform, the trial ended. If an animal failed to find the platform within 60 seconds, it was placed on the platform for 15 seconds, then returned to the cage. The latency to reach the platform was measured by stopwatch and the route was automatically tracked as in the Open field.

Learning: With the platform situated 1cm below the surface at the center of the SW quadrant, animals were trained until a stable performance was established (5 blocks of 4 trials on 5 consecutive days.

Memory: Three weeks later, animals were tested again with the platform in the same location (2 blocks of 4 trials on 2 consecutive days).

New platform position I: The day after the memory test, animals were tested in a reversal procedure with platform opposite the original location, now at the center of NE quadrant. (2 blocks of 4 trials on 2 consecutive days).

New platform position II: On the following day, the platform was located in the center of the pool (1 block of 4 trials).

Results

NOAEL maternal toxicity: 500ppm

LOAEL developmental neurotoxicity: 500ppm [impairment of learning and memory performance by female offspring]

NOAEL developmental neurotoxicity: None [one dose only]

Maternal effects: No adverse effects were observed for maternal weight gain, food consumption, pregnancy length, and number of pups per litter, or sex distribution in the litters.

Neonatal growth: Neonatal deaths and preimplantation loss were not statistically significantly different between control and xylene exposed groups.

The incidence of postimplantation loss was higher in control (7.1%) than in exposed group (2.8%) while more pups died postnatally in the exposed group (0.5 pups/litter; 7 pups among 15 litters) compared to controls (0.2 pups/litter; 3 pups among 13 litters). Mean birth weight was slightly but not statistically significantly lower in pups of the exposed group (5.9g) compared to controls (6.3g). No significant exposure related differences in offspring body weight were seen during lactation and the postweaning period. Average absolute brain weight determined from one weanling pup/sex/litter on PND 28 was significantly lower in the exposed group, which correlated with a trend toward lower body weight. Brain weight relative to body weight was comparable in both groups.

Neonatal development

Physical development and time course for development of reflexes were similar except for a statistically significant delayed appearance of air righting reflex in exposed litters on PND 16. The 4 pups from 4 xylene-exposed litters, which failed to air right on PND 16 were successful when retested on PND 17. Females were affected more than males.

Neonatal Neurobehavioral tests

Rotarod: Differences between pups exposed to xylene *in utero* and control pups were not statistically significant. The percentage of animals not meeting the 30-second criterion was higher in the exposed group on all three testing days and the mean time on the rotarod was shorter. Again, females were more affected than males, especially on PND 26 trials. [Effects on rotarod performance were not as significant as reported at 200ppm xylene in the earlier Hass and Jakobsen 1993 study.]

Open Field: No exposure-related differences were observed.

Morris Water Maze: All animals could swim easily with the characteristic adult swimming posture. Housing appeared to influence performance especially in the beginning of the period. No differences in latency were found in enriched-housed pups compared to controls while standard housed pups from the xylene-exposed group showed a slightly non-significant trend in the time needed to find the platform in the beginning of the learning part of the test. This effect on standard-housed offspring at 12 wks [3 months] could be attributed to a slight increase in latency during the first 2 trials of a 5-trial learning phase with no difference in learning during the last 3 trials. The absence of effect on learning in enriched-housed rats suggests that any effect of prenatal xylene exposure was, at least partially, overcome by the enriched housing environment. No differences were seen between either housed exposure groups or controls when testing for memory 3 weeks later [approximately week 16] or in reversal learning when the platform was moved to the opposite quadrant of the pool. However, when

the platform was relocated in the center of the pool requiring a mapping strategy by the animal to find the platform, the offspring of the xylene exposed group used significantly more time, when data from the sexes were combined. Analysis by sex indicated differences were significant only in female offspring, who also revealed a significantly increased swimming length. No differences in swim speed were observed between exposed or control offspring.

In a follow-up study to assess persistence of the decreased water maze performance, these same female offspring raised in standard housing were maintained and evaluated at 28 and 52 wks. (Hass et al, 1997). At 28 weeks, an increased latency in finding the platform in the center was not repeated although there was an effect on latency when the platform was placed in a new position. However no other significant differences were observed for other testing situations in the water maze and no statistically significant differences of any kind were observed at 55 wks.

Conclusions (study authors)

Prenatal exposure of rats to 500ppm technical xylene caused statistically significant impaired performance in behavioral tests for learning and memory (Morris Water maze). Effects were most marked in female offspring. Other effects seen were delayed ontogeny of air righting reflex, a lower absolute brain weight coincident with a trend for lower body weight but not significant relative to body weight in selected pups and a non statistically significant impaired performance in the rotarod neuromotor test.

Data Quality Reliabilities

2. Reliable with restrictions. Only a single dose was employed. The number of pregnant females at the initiation of exposure and the number of sperm-positive females without litters were not reported. Use of GLPs was not specified.

References

Hass, U., Lund, S.P., Simonsen, L., and Fries, A.S. 1995. Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotox Teratol 17: 341-349.

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Hass, U., Lund, S.P., and Simonsen, L. 1997. Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. *Neurotoxicology* 18: 547-551.

Nelson, C.J., Felton, R.P., Kimmel, C.A. et al., 1985. Collaborative behavioral teratology study: Statistical approach. Neurobehav Toxicol Teratol 7: 487-590

Other

Last changed 9/08/2005