The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Xylene (all isomers)

MAK Value Documentation, supplement – Translation of the German version from 2021

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

xylene; fertility; developmental toxicity; developmental neurotoxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the developmental toxicity of xylene [1330-20-7]. In 2019, the maximum concentration at the workplace (MAK value) of xylene was lowered to 50 ml/m^3 after taking into account the increased respiratory volume at the workplace (see List of MAK and BAT Values, Section Ib and Ic). The critical effect of xylene is acute neurotoxicity. Due to mixed exposure and the limited validity of epidemiologic studies, no conclusions can be drawn with respect to developmental toxicity in humans. In animal studies, xylene did not demonstrate a teratogenic potential. Significant decreases in foetal body weights were observed in rats in prenatal toxicity studies after inhalation exposure to the *o*-isomer or the technical mixture at a concentration of 500 ml/m^3 and above; the NOAEC was 100 ml/m^3. This effect was observed with the *m*- and *p*-isomers only at higher concentrations. Maternal toxicity in the form of reduced maternal body weight gains was observed at 1000 ml/m^3 and 2000 ml/m^3 after exposure to all isomers and the mixture, respectively. The littermates of pregnant rats exposed to *p*-xylene at concentrations up to 1589 ml/m^3 from days 7 to 16 of gestation did not show treatment-related effects on acoustic startle response or figure-8 maze activity. As the *o*-isomer proved to be more potent in lowering foetal body weights, but was not examined in the aforementioned study, and as comprehensive data are not available for the developmental toxicity of the isomers and the mixture, the classification of xylene in Pregnancy Risk Group D has been retained.

Citation Note:
Hartwig A, MAK Commission. Xylene (all isomers). MAK Value Documentation, supplement – Translation of the German version from 2021. MAK Collect Occup Health Saf. 2022 Sep;7(3):Doc053. https://doi.org/10.34865/mb133020be7_3ad

Manuscript completed:
14 Apr 2020

Publication date:
30 Sep 2022

MAK value (2019)	**50 ml/m³ (ppm) ≙ 220 mg/m³**
Peak limitation (2001)	**Category II, excursion factor 2**
Absorption through the skin (1998)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (1988)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value (1984)	**2000 mg methylhippuric acid/l urine**
Vapour pressure	**xylene (CAS number: 1330-20-7)** 8.2 hPa at 20 °C (calculated) (ECHA 2020 d) ***o*-xylene (CAS number: 95-47-6)** 7 hPa at 20 °C (exp.) (NCBI 2020 b) 13.4 hPa at 32.2 °C (exp.) (ECHA 2020 b; NCBI 2020 b) ***m*-xylene (CAS number: 108-38-3)** 8 hPa at 20 °C (exp.) (NCBI 2020 a) 11.1 hPa at 25 °C (exp.) (ECHA 2020 a; NCBI 2020 a) ***p*-xylene (CAS number: 106-42-3)** 9 hPa at 20 °C (exp.) (NCBI 2020 c) 11.7 hPa at 25 °C (exp.) (ECHA 2020 c) 13.1 hPa at 25 °C (exp.) (NCBI 2020 c)
1 ml/m³ (ppm) ≙ 4.41 mg/m³	**1 mg/m³ ≙ 0.227 ml/m³ (ppm)**

In 2019, the MAK value was lowered from 100 ml/m³ to 50 ml/m³ after taking into account the increased respiratory volume of humans at the workplace (Hartwig and MAK Commission 2022). This supplement revises the assignment to a pregnancy risk group for the same reason. Most of the data used for evaluating developmental toxicity were already available in the 1983 documentation (Henschler 1993), the 1998 supplement (Greim 2001) and the 2004 supplement (Hartwig 2014).

Effects in Humans

Reproductive and developmental toxicity

Fertility

In a cross-sectional study of 1408 workers from petrochemical plants in China, the influence of exposure to organic solvents such as benzene, styrene, toluene or xylene on the menstrual cycle was investigated. Of the workers, 440 were exposed to solvents. The mean exposure level for toluene, styrene and xylene was lower than 1 ml/m³. No workers were exposed to xylene alone. After adjustment for confounding factors, a 53% increase in the incidence of oligomenorrhoea (odds ratio (OR) 1.53; 95% confidence interval (CI): 1.00–2.34) was found after 3 years or more of exposure to the solvent

mixture (Cho et al. 2001; Hartwig and MAK Commission 2022). Due to the exposure to a mixture of substances, the results of this study cannot be evaluated with regard to xylene.

Seminal fluid and blood samples were collected between June 1994 and July 1996 from 24 married male employees living in a city in Zhejiang Province, China, who had been occupationally exposed to benzene, toluene and xylene for at least 1 year. Age-matched workers not exposed to the substances were used as controls. In the exposed workers, sperm vitality and motility were reduced compared with the findings in the controls; in the sperm, the mean acrosin activity, γ-glutamyl transferase activity and the relative lactate dehydrogenase-C4 activity were decreased, and the fructose concentration was increased. The mean concentrations at the workplace were 103.34 mg benzene/m^3 (0–7070.3 mg/m^3), 42.73 mg toluene/m^3 (0–435.8 mg/m^3) and 8.21 mg xylene/m^3 (0–133.1 mg/m^3) (Xiao et al. 2001). Due to the exposure to a mixture of substances, this study can likewise not be used for the evaluation.

Developmental toxicity

A case–control study in Finland investigated the relationship between the incidence of spontaneous abortions and exposure to solvents at the workplace. The study population was identified using three sources: 1) the register of female employees who were biologically monitored for solvent exposure from 1965–1983 by the Institute of Occupational Health; 2) the national pregnancy database in Finland; 3) the Finnish Register of Congenital Malformations. Spontaneous abortions made up 8.9% of all pregnancies in 1973–1983. "Cases" were those workers exposed to organic solvents who had a spontaneous abortion (n = 120). Controls were workers exposed to organic solvents who had no spontaneous abortions and no children with congenital malformations (n = 336). Exposure levels were classified on the basis of the activity carried out, job description and solvent use reported by the pregnant workers and by measured biological data, if available. In 21 pregnant workers who occupied the same job during their pregnancy and were investigated, the measure of xylene exposure was a methylhippuric acid concentration of 0.7 ± 0.4 mmol/l urine. For xylene, there were 5 exposed cases and 7 exposed controls. The OR for spontaneous abortions was 1.3 (95% CI: 0.4–4.5). The logistic regression model also took into account previous spontaneous abortions, parity, smoking, alcohol consumption, and exposure to other solvents (Lindbohm et al. 1990). The number of cases is too small to assess the developmental toxicity potential of xylene.

In another publication by the same working group, the relationship between the laboratory activities of female employees and spontaneous abortions, congenital malformations, and body weights of the offspring was investigated. The study participants were drawn from three sources: 1) the list of state-employed laboratory personnel in Finland; 2) the Finnish Union of Laboratory Assistants; 3) the register of employees exposed to carcinogens. The study of spontaneous abortions included 535 women (n = 206, controls: 329) and that of malformations 141 women (n = 36, controls: 105). The analysis of birth weights included the offspring of 500 women. Exposure was assessed by questionnaires, and an exposure index was calculated. Adjustments were made for smoking, alcohol consumption, medication, febrile illness and employee status as confounders. In the case of xylene, there was a statistically significant association with spontaneous abortion for employment for at least 3 days per week with an OR of 3.1 (95% CI: 1.3–7.5; 16 cases, 12 controls). An association with congenital malformations was not found. Statistically significant changes in birth weights were also not observed (Taskinen et al. 1994). The number of cases is likewise too small to draw any conclusion on the developmental toxicity potential of xylene.

A case–control study in Texas examined the relationship between the estimated maternal exposure to benzene, toluene, ethylbenzene and xylene at the place of residence and the risk of orofacial clefts in offspring. Data for 6045 offspring with non-syndromic isolated orofacial clefts (3915 cleft lips with or without cleft palate and 2130 cleft palates) born between 1999 and 2008 were obtained from the Texas Birth Defects Registry. The control group was a sample of unaffected live births. Census tract-level estimates of annual average exposures were obtained from the U.S. Environmental Protection Agency 2005 Hazardous Air Pollutant Exposure Model for each substance and assigned to each subject based on maternal residence during pregnancy. To assess the relationship between the estimated maternal exposure to each substance and the risk of orofacial clefts in the offspring, a logistic regression model was used. ORs were adjusted for the year of birth, sex, maternal ethnicity, education level, age and smoking. For the high xylene

concentration of at least 1.28 µg/m^3 estimated by the model, the adjusted ORs were 0.93 (95% CI: 0.80–1.10) for cleft lip with or without cleft palate, and 0.85 (95% CI: 0.66–1.08) for cleft palate. There was no evidence that maternal exposure to xylene or to the other substances was associated with the occurrence of this malformation (Ramakrishnan et al. 2013).

Another study calculated the risk for autism in children after in utero exposure to monitored ambient air pollutants from urban emissions. The cohort included children (n = 148 722) from Los Angeles County, California, between 1995 and 2006 whose mothers resided during pregnancy within a 5 km radius around air pollutant monitoring stations. These birth records were linked to the records of the California Department of Developmental Services for diagnosed primary autistic disorders between 1998 and 2009 (n = 768). Monthly average exposures during pregnancy were calculated for 24 air pollutants suspected or known to cause neurotoxicity or developmental neurotoxicity. Factor analysis was used to identify correlating structures among air pollutants. ORs were calculated using logistic regression. Autism risks were increased when the interquartile range of the average concentrations during pregnancy of several correlated substances was increased; this was mostly due to one air pollutant. For *m*-xylene/*p*-xylene, the OR was 1.51 (95% CI: 1.26–1.82). Adjustment was made for maternal age, ethnicity, education, insurance type, maternal birthplace, parity, sex of the child and birth year. The authors themselves reported several limitations of the study, such as the non-differential misclassification of exposure and unexplained diagnostic differences among regional centres. In addition, no information was given for the smoking status of the mothers (von Ehrenstein et al. 2014).

In a study, the relationship between perinatal exposure to air pollutants and retinoblastoma formation was investigated. Cases diagnosed between 1990 and 2007 were ascertained from California Cancer Registry records of children and matched to Californian birth certificates. Controls were randomly selected from the state birth registry for the same period. The study included 103 cases (30 601 controls) living within a 5 mile radius of an air pollution monitoring site. Twenty-seven air pollutants were selected for the study that were listed by the IARC as possible, probable or established human carcinogens. Logistic regression analyses were used to model the risk of retinoblastomas due to exposure to air pollutants, separately for exposure during pregnancy and in the first year of life. As the interquartile range of exposure to air pollutants increased, an increased risk for retinoblastoma formation was found for 6 air pollutants, including *o*-xylene and *m*-xylene/*p*-xylene. The ORs for exposure during pregnancy to these substances were 1.44 (95% CI: 1.06–1.96; cases: 81, controls: 22 247) and 1.37 (95% CI: 1.02–1.83; cases: 56, controls: 15 718), respectively. The mean concentrations and standard deviations were 0.50 ± 0.33 and 1.05 ± 0.59 ppbV (parts per billion by volume, 10^{-7} vol%), respectively. Adjustment was made for maternal ethnicity, nativity, paternal age, year of birth and socioeconomic status. Adjustment for smoking was not possible because smoking status data were not recorded in the National Birth Registry before 2007 (Heck et al. 2015). The validity of the study is, in addition, significantly limited due to the lack of data for medication, and alcohol and drug consumption during pregnancy. It is known that prenatal ingestion of these substances can induce damage to the retina or other parts of the eye (Peragallo et al. 2013).

A study in Mexico investigated the relationship between exposure to outdoor and indoor air pollutants during pregnancy and the cognitive development trajectories of the first 7 years of life. It investigated 718 mother–infant pairs from the programme of prenatal omega-3 supplementation reported on by this research group in 2010. Prenatal exposure to indoor pollutants (mould, ventilation, pesticides, tobacco smoke and the use of handmade clay pots) was self-reported by the mothers and integrated into an index or objectively measured (including the determination of xylene, nitrogen oxides, benzene and toluene) in the case of outdoor air. Children's cognitive development was determined at 12, 18, 60 and 84 months using Latent Class Growth Analysis and three different developmental trajectories were identified (positive = 108, average = 362, low = 248). Using a multinomial logistic model, associations were calculated between the Environmental Pollutant Score (EPS) or outdoor air pollutants and cognitive development trajectories. After adjusting for sociodemographic covariates (obstetric information, anthropometric measurements, diet, schooling and education, maternal intelligence, breastfeeding, stimulation and learning environment, school attendance and sex), an association for EPS with average (OR = 1.26; 95% CI: 1.01–1.55) and low (OR = 1.41; 95% CI: 1.11–1.79) cognitive developmental trajectories, where a unit increase in EPS means an additional prenatal exposure to a pollutant, was calculated. There was no correlation between outdoor air pollutants and cognitive development trajectories. The authors concluded that the children of mothers exposed to indoor environmental pollutants during pregnancy were more likely to follow a poorer cognitive developmental trajectory during the first 7 years of life. The mean outdoor xylene concentration in

air was 3.5 ± 5.2 µg/m³ (Gonzalez-Casanova et al. 2018). The development of cognitive abilities is a complex process that is influenced by many factors, both genetic and external. External factors not mentioned in the study are, for example, alcohol, medication intake and viral infections during pregnancy.

A study of exposure to volatile organic compounds was conducted in pregnant women as part of the National Children's Study in the USA. Biomonitoring, including the determination of metabolites of xylene in urine, was performed, but no information was provided on the course/outcome of the pregnancy (Boyle et al. 2016).

Conclusion: The studies are not suitable for evaluating the developmental toxicity of xylene in humans due to their limited validity and the exposure to a mixture of substances.

Animal Experiments and in vitro Studies

Reproductive and developmental toxicity

Fertility

In a one-generation study, male and female CD rats, were exposed by inhalation to technical xylene concentrations of 0, 60, 250 or 500 ml/m³ (technical xylene: 2.4% toluene, 12.8% ethylbenzene, 20.3% *p*-xylene, 44.2% *m*-xylene, 20.4% *o*-xylene) for 6 hours daily on 5 days per week, 131 days before mating, during gestation, and during lactation from day 5 to day 20. Two other groups included animals of only one sex exposed to 500 ml/m³. No adverse effects were noted in the F0 adults. Histological examination of the reproductive organs did not yield any unusual findings and the testis weights were unchanged. Although the female mating index was lower in a statistically significant manner (85% in both groups) at 250 ml/m³ (both sexes exposed) and 500 ml/m³ (only females exposed) than in the controls (100%), this was not considered to be substance-related as no effect was observed when both sexes were exposed to 500 ml/m³, and the control animals had an unusually high mating performance. The male mating index, pregnancy incidence and fertility index were comparable to the control values. The NOAEC (no observed adverse effect concentration) for parental toxicity and fertility was 500 ml/m³, the highest concentration tested (see also Section "Developmental toxicity") (US EPA 2003).

Developmental toxicity

In the 1983 documentation (Henschler 1993), it was reported that teratogenic effects of xylene were noticed in 4 experimental studies in animals (Hudák and Ungváry 1978; Krotov and Chebotar' 1972; Ungváry et al. 1980, 1981). The studies in question described extra ribs and fused sternebrae in CFY rats exposed to xylene by inhalation (Hudák and Ungváry 1978; Ungváry et al. 1980). According to the Devtox Database ("ribs, supernumerary site", "sternebrae, fused"; BfR 2020), both changes occurred in the grey area between variations and malformations. In the other two studies, no malformations are mentioned in the table given in the 1983 documentation. The absence of malformations has been confirmed by a re-examination of these studies (Krotov and Chebotar' 1972; Ungváry et al. 1981). Overall, these studies do not provide evidence of a teratogenic effect of xylene in rats.

In the 1998 supplement (Greim 2001), another study in mice is mentioned in which the number of foetuses with malformations was increased. Doses of technical xylene of 0, 0.6, 1.2, 2.4, 3.0, 3.6 or 4.8 ml/kg body weight and day were administered by gavage (vehicle: cottonseed oil) 3 times daily to groups of 15 to 28 CD-1 mice (control group: 66 animals) from days 6 to 15 of gestation. This corresponded to 0, 520, 1030, 2060, 2580, 3100 or 4130 mg/kg body weight and day at a density of 0.86 g/ml and via correction of the unit misreported in the study. The technical xylene consisted of 60.2% *m*-xylene, 9.1% *o*-xylene, 13.6% *p*-xylene and 17.0% ethylbenzene. At 3100 mg/kg body weight and day, only 26 of 38 animals were alive on day 18 of gestation, at 4130 mg/kg body weight and day all 15 dams had died, and in the other dose groups none of the animals died. At 3100 mg/kg body weight and day, maternal body weight gains were decreased. At 2060 mg/kg body weight and day and above, marginally increased liver weights were observed

in the dams. An increased number of foetuses with external malformations was observed at these dose levels, with cleft palate being the most common. At the same time, the body weights of the foetuses were decreased. In addition, wavy ribs occurred at 2580 and 3100 mg/kg body weight and day. Cleft palate (0, 520, 1030, 2060, 2580, 3100 mg/kg body weight and day: 0/658, 0/208, 1/232, 6/227, 11/224, 7/79) and open eyes (bilateral, 0/658, 0/208, 0/232, 1/227, 0/224, 0/79) that occurred at 2060 mg/kg body weight and day and above were regarded as malformations because they were not the result of the statistically significant decrease in mean maternal body weights. There was no statistically significant change in the number of implantations at any dose (Marks et al. 1982). A NOAEL (no observed adverse effect level) for developmental toxicity of 1030 mg/kg body weight and day can be derived from this study (Greim 2001). The doses used were extremely high, exceeding the limit dose in the OECD test guidelines of 1000 mg/kg body weight and day even with the second lowest dose of 1030 mg/kg body weight and day. The induction of cleft palates in mice must be viewed very critically; a stress mechanism could have taken effect here, especially at such high doses. Such malformations have not been observed in a second species.

In another study in CFY rats, CFLP mice and New Zealand White rabbits, technical xylene (no details of composition) and the isomers *o*-xylene, *m*-xylene and *p*-xylene were studied. Groups of 19 to 23 rats were exposed to technical xylene concentrations of 0, 250, 1200 or 3400 mg/m^3 (24 hours daily, days 7 to 15 of gestation, or 2 to 4 hours daily, day 18 or 20 of gestation), groups of 15 to 18 mice to 0 or 500 mg *o*-xylene, *m*-xylene or *p*-xylene/m^3 or 0, 500 or 1000 mg technical xylene/m^3 (24 hours daily, days 6 to 15 of gestation) and groups of 9 to 10 rabbits were exposed whole-body to 0, 500 or 1000 mg *o*-xylene, *m*-xylene, *p*-xylene or technical xylene/m^3 (24 hours daily, days 6 to 15 of gestation). The control animals were exposed to air. Maternal toxicity was described as moderate and concentration-dependent but was not otherwise specified. In rats, retarded skeletal ossification occurred at technical xylene concentrations of 250 mg/m^3 and above. At a technical xylene concentration of 3400 mg/m^3, the number of dead or resorbed foetuses and foetuses with extra ribs was increased. Mice exhibited retarded skeletal ossification and delayed body weight gains with all test substances. In rabbits, increased numbers of dead or resorbed foetuses occurred at the *m*-xylene concentration of 500 mg/m^3. All compounds produced abortions in rabbits at 1000 mg/m^3. Teratogenic effects were not observed in any of the species tested (Ungváry and Tátrai 1985). Since the presentation and description of the results is deficient, the study is not included in the evaluation.

In a review, it was concluded that the *o*-isomer and *p*-isomer pose a greater risk to the embryo than the *m*-isomer (Hood and Ottley 1985).

In the one-generation study described above, also the examination of external, visceral and skeletal malformations/variations of the foetuses on day 21 of gestation was carried out. No abnormalities were found and a NOAEC of 500 ml/m^3 was derived for maternal and developmental toxicity (see also Section "Fertility") (US EPA 2003).

Studies with behavioural experiments in the offspring of dams treated during gestation (Hass et al. 1995, 1997; Hass and Jakobsen 1993) have not yet been evaluated. A detailed review of these studies can be found in Table 1.

In a study in Mol:WIST rats, both developmental toxicity and postnatal behavioural experiments were performed after whole-body exposure for 6 hours daily from days 4 to 20 of gestation. Delayed ossification of the os maxillare occurred in foetuses at the only concentration tested of 200 ml technical xylene/m^3. Maternal toxicity was not observed. Ear unfolding and eye opening occurred earlier in the offspring, possibly due to the higher body weights. The performance in the rotarod test was impaired at 200 ml technical xylene/m^3 on some days. However, the experiment was not performed blind (Hass and Jakobsen 1993). The use of the rotarod test in rats is considered problematical because their "motivation" to run is often impaired. This is reflected in occasional abnormal falls, irregular behaviour and poor overall rotarod performance (Deacon 2013; Rozas et al. 1997). Therefore, the rotarod test in rats does not provide unambiguous information. In addition, the behavioural experiment was not conducted blind and must therefore be regarded as not valid.

This research group conducted further behavioural tests in the same species with inhalation exposure in whole-body chambers from days 7 to 20 of gestation, but these studies were carried out blind. Maternal toxicity was not observed in either study (Hass et al. 1995, 1997). At the only concentration tested of 500 ml technical xylene/m^3, reduced performance in the rotarod test was seen in the offspring, but this was not statistically significant in pairwise comparisons

with the controls. In the Morris water maze test, only females in one subgroup needed more time to find the platform and their swimming length was increased compared with that of the controls when the platform was relocated. The subgroup consisted of the animals reared in a standard environment (Hass et al. 1995). The effect in the Morris water maze test is considered marginal because it occurred only when the platform was relocated and also only in one subgroup and in one sex. The following experiment describes additional time points in the Morris water maze test with the same females. When the platform was relocated, an effect was observed at the age of 28 weeks but not at 55 weeks (Hass et al. 1997). It was not apparent which animals were tested and when. Moreover, the effects were observed only on some days and also only in one sex. The subgroups reared in the standard or enriched environment from the 1995 study were not mentioned in the follow-up study.

In all three studies, only one concentration was tested. Thus, the analysis of a concentration–response relationship is not possible. The behavioural tests were not performed according to valid test guidelines. Therefore, the behavioural tests of the three studies (Hass et al. 1995, 1997; Hass and Jakobsen 1993) are not included in the evaluation.

In another previously described study involving a behavioural experiment, groups of 25 Sprague Dawley rats were exposed whole-body to *p*-xylene concentrations of 0, 3500 or 7000 mg/m³ (795 or 1589 ml/m³, respectively) (purity: at least 99%) for 6 hours per day from days 7 to 16 of gestation. At the *p*-xylene concentration of 1589 ml/m³, the body weight gains of the dams were reduced. On postnatal day 4, the litters were reduced to 8 offspring each (4 per sex). Ten litters per concentration group were used for the behavioural tests. The litter size, birth weights, acoustic startle response measured on postnatal days 13, 17, 21 and 63, and locomotor activity determined by the figure-8 maze test on postnatal days 22 and 65 were unchanged compared with the values in the controls (Greim 2001; Rosen et al. 1986). The NOAEC for maternal toxicity was 795 ml *p*-xylene/m³, the NOAEC for effects on the acoustic startle response and locomotor activity was 1589 ml/m³, the highest concentration tested.

Tab. 1 Developmental toxicity studies with xylene from the Hass research group

Species, strain, number per group	Exposure	Findings	References
rat, Mol:WIST, 22 ♀ controls, 25 ♀	**GD 4–20**, 0, 200 ml/m³, inhalation, whole-body, 6 hours/day, purity: technical xylene (not further specified), examined on: GD 22, investigated parameters similar to OECD Test Guideline 414	**200 ml/m³**: foetuses: delayed ossification of the os maxillare (18/26 litters, controls: 2/22); no maternal toxicity	Hass and Jakobsen 1993
rat, Mol:WIST, 10 ♀ controls, 9 ♀	**GD 4–20**, 0, 200 ml/m³, inhalation, whole-body, 6 hours/day, purity: technical xylene (not further specified), litters reduced to 8 offspring after birth (4 per sex where possible), **investigation of postnatal development (offspring from 1/3 of the dams)**: unfolding of ears (from PND 3), surface righting reflex (from PND 3 onwards), cliff avoidance reflex (PND 7), incisor eruption (PND 9), opening of eyes (from PND 13 onwards), auditory startle reflex (PND 13) and rotarod test (PND 22, 23, 24), not performed blind	**200 ml/m³**: offspring: body weights ↑ (PND 0: ♂: 6.6 ± 0.4 g; controls 6.1 ± 0.4 g; PND 28: ♂: 82 ± 4 g; controls: 73 ± 6 g; ♀: 76 ± 2 g; controls: 71 ± 4 g); earlier ear unfolding and eye opening (possibly due to higher body weights), performance in rotarod test ↓ (all offspring of relevant litters tested; ♂: PND 23, ♀: PND 22, PND 23, PND 24); no maternal toxicity	Hass and Jakobsen 1993

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Mol:WIST, 13 ♀ controls, 15 ♀	**GD 7–20**, 0, 500 ml/m³, inhalation, whole-body, 6 hours/day, purity: technical xylene (19% *o*-xylene, 45% *m*-xylene, 20% *p*-xylene, 15% ethylbenzene), litters with less than 6 offspring not included in the study of postnatal development, **investigation of postnatal development**: before weaning (all offspring): ear unfolding (from PND 2 onwards), surface righting (from PND 2 onwards), homing response (PND 6 and 7), incisor eruption (from PND 10 onwards), auditory startle reflex (from PND 12), eye opening (from PND 13 onwards), air righting (from PND 15 onwards); after weaning: rotarod test (PND 24-26, 1 animal per sex and litter), open field (PND 27, PND 32 ± 2, 1 animal per sex and litter), sexual maturity (from PND 30 onwards, 1 animal per sex and litter), Morris water maze (3–4 months, 2 animals per sex and litter), behavioural experiments performed blind	**500 ml/m³**: offspring: delayed righting reflex (PND 15, PND 16; only ♀, 4 affected offspring re-tested on PND 17: effect reversible), absolute brain weights ↓ (PND 28 ± 2; ♂, ♀; relative brain weights not changed), performance in rotarod test ↓ (2 test criteria: percentage of animals that could not stay on the rod for 30 seconds; average time on the rod; no statistical significance in pairwise comparison with controls), Morris water maze ↓ (♀: platform relocation: time to find platform and swimming length ↑ compared with values in the controls, statistically significant only in animals reared in the standard environment, not statistically significant in animals reared in the enriched environment), ♀ more sensitive; no maternal toxicity; marginal effect in the Morris water maze test and only in one subgroup	Hass et al. 1995
rat, Mol:WIST, 13 ♀ controls, 15 ♀, the same animals as in the study of 1995, animals monitored in recovery period up to week 55	**GD 7–20**, 0, 500 ml/m³, inhalation, whole-body, 6 hours/day, purity: technical xylene (19% *o*-xylene, 45% *m*-xylene, 20% *p*-xylene, 15% ethylbenzene), **investigation of postnatal development**: Morris water maze (12–16, 28 and 55 weeks, 1 ♀ tested each time with average body weight per litter), behavioural experiment performed blind	**500 ml/m³**: offspring: Morris water maze ↓ (♀: 12–16 and 28 weeks: platform relocated: time to find platform and swimming length ↑ compared with the values in the controls, not statistically significant at 55 weeks); no maternal toxicity; unclear as to which animals were tested and when	Hass et al. 1997

GD: gestation day; PND: postnatal day

Manifesto (MAK value/classification)

The critical effects of xylene are its acute neurotoxic effects in humans and in animal studies.

Prenatal toxicity. Due to the exposure to a mixture of substances and the limited validity of the studies in humans, these studies are not suitable for evaluating the developmental toxicity of xylene.

Xylene was not found to be teratogenic in animal studies. In a prenatal developmental toxicity study in Sprague Dawley rats, exposure to *o*-xylene and technical xylene at concentrations of 500 ml/m³ and above led to decreased foetal body weights. The NOAEC for this effect was 100 ml/m³. The other isomers caused this effect only at higher concentrations. In addition, an increase in skeletal variations was found with *o*-xylene at and above 1000 ml/m³ and with *m*-xylene and *p*-xylene at 2000 ml/m³. The NOAEC for maternal toxicity based on delayed body weight development and reduced feed intake was 100 ml/m³ for all isomers and 500 ml/m³ for technical xylene (Hartwig 2014; Saillenfait et al. 2003).

Since the MAK value has been derived on the basis of a neurotoxic effect, a statement on developmental neurotoxicity must be made. However, only one study is available that can be used for the evaluation. In this study, with prenatally exposed Sprague Dawley rats, no effects were found up to the highest concentration tested of 1589 ml *p*-xylene/m³ with regard to the acoustic startle response and locomotor activity in the postnatal period (Greim 2001; Rosen et al. 1986). However, as the *o*-isomer proved to be more potent than the *p*-isomer in lowering foetal body weights (Saillenfait

et al. 2003), the *o*-isomer in the study by Rosen et al. (1986) was not investigated, and comprehensive developmental neurotoxicity studies are not available for the various isomers, the classification of xylene in Pregnancy Risk Group D has been retained.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

BfR (Bundesinstitut für Risikobewertung) (Federal Institute for Risk Assessment) (2020) DevTox: a resource for developmental toxicology. https://www.devtox.org/nomenclature/ml_organ.php?lan=en, accessed 16 Sep 2020

Boyle EB, Viet SM, Wright DJ, Merrill LS, Alwis KU, Blount BC, Mortensen ME, Moye J Jr, Dellarco M (2016) Assessment of exposure to VOCs among pregnant women in the National Children's Study. Int J Environ Res Public Health 13(4): 376. https://doi.org/10.3390/ijerph13040376

Cho S-I, Damokosh AI, Ryan LM, Chen D, Hu YA, Smith TJ, Christiani DC, Xu X (2001) Effects of exposure to organic solvents on menstrual cycle length. J Occup Environ Med 43(6): 567–575. https://doi.org/10.1097/00043764-200106000-00012

Deacon RMJ (2013) Measuring motor coordination in mice. J Vis Exp (75): e2609. https://doi.org/10.3791/2609

ECHA (European Chemicals Agency) (2020 a) m-Xylene (CAS Number 108-38-3). Registration dossier. Joint submission, first publication 17 Feb 2011, last modification 28 Apr 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14230, accessed 04 May 2020

ECHA (European Chemicals Agency) (2020 b) o-Xylene (CAS Number 95-47-6). Registration dossier. Joint submission, first publication 03 Mar 2011, last modification 18 Sep 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15482, accessed 04 May 2020

ECHA (European Chemicals Agency) (2020 c) p-Xylene (CAS Number 106-42-3). Registration dossier. Joint submission, first publication 03 Mar 2011, last modification 28 Apr 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15791, accessed 04 May 2020

ECHA (European Chemicals Agency) (2020 d) Xylene (CAS Number 1330-20-7). Registration dossier. Joint submission, first publication 17 Mar 2011, last modification 28 Apr 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15448, accessed 04 May 2020

von Ehrenstein OS, Aralis H, Cockburn M, Ritz B (2014) In utero exposure to toxic air pollutants and risk of childhood autism. Epidemiology 25(6): 851–858. https://doi.org/10.1097/EDE.0000000000000150

Gonzalez-Casanova I, Stein AD, Barraza-Villarreal A, Feregrino RG, DiGirolamo A, Hernandez-Cadena L, Rivera JA, Romieu I, Ramakrishnan U (2018) Prenatal exposure to environmental pollutants and child development trajectories through 7 years. Int J Hyg Environ Health 221(4): 616–622. https://doi.org/10.1016/j.ijheh.2018.04.004

Greim H, editor (2001) Xylene (all isomers). MAK Value Documentation, 1998. In: Occupational Toxicants. Volume 15. Weinheim: Wiley-VCH. p. 257–288. Also available from https://doi.org/110.1002/3527600418.mb133020e0015

Hartwig A, editor (2014) Xylene (all isomers). MAK Value Documentation, 2001. In: The MAK Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb133020e3314

Hartwig A, MAK Commission (2022) Xylene (all isomers). MAK Value Documentation, supplement – Translation of the German version from 2020. MAK Collect Occup Health Saf 7(3): Doc052. https://doi.org/10.34865/mb133020ae7_3ad

Hass U, Jakobsen BM (1993) Prenatal toxicity of xylene inhalation in the rat: a teratogenicity and postnatal study. Pharmacol Toxicol 73(1): 20–23. https://doi.org/10.1111/j.1600-0773.1993.tb01951.x

Hass U, Lund SP, Simonsen L, Fries AS (1995) Effects of prenatal exposure to xylene on postnatal development and behavior in rats. Neurotoxicol Teratol 17(3): 341–349. https://doi.org/10.1016/0892-0362(94)00093-s

Hass U, Lund SP, Simonsen L (1997) Long-lasting neurobehavioral effects of prenatal exposure to xylene in rats. Neurotoxicology 18(2): 547–551

Heck JE, Park AS, Qiu J, Cockburn M, Ritz B (2015) Retinoblastoma and ambient exposure to air toxics in the perinatal period. J Expo Sci Environ Epidemiol 25(2): 182–186. https://doi.org/10.1038/jes.2013.84

Henschler D, editor (1993) Xylene (all isomers). MAK Value Documentation, 1983. In: Occupational Toxicants. Volume 5. Weinheim: VCH. p. 263–285. Also available from https://doi.org/10.1002/3527600418.mb133020e0005

Hood RD, Ottley MS (1985) Developmental effects associated with exposure to xylene: a review. Drug Chem Toxicol 8(4): 281–297. https://doi.org/10.3109/01480548509038651

Hudák A, Ungváry G (1978) Embryotoxic effects of benzene and its methyl derivatives: toluene, xylene. Toxicology 11(1): 55–63. https://doi.org/10.1016/s0300-483x(78)90439-0

Krotov IA, Chebotar' NA (1972) [Study of the embryotoxic and teratogenic effect of several industrial substances formed during the production of dimethylterephthalate]. Gig Tr Prof Zabol 16(6): 40–43

Lindbohm M-L, Taskinen H, Sallmén M, Hemminki K (1990) Spontaneous abortions among women exposed to organic solvents. Am J Ind Med 17(4): 449–463. https://doi.org/10.1002/ajim.4700170404

Marks TA, Ledoux TA, Moore JA (1982) Teratogenicity of a commercial xylene mixture in the mouse. J Toxicol Environ Health 9(1): 97–105. https://doi.org/10.1080/15287398209530145

NCBI (National Center for Biotechnology Information) (2020 a) m-Xylene. PubChem compound summary for CID 7929. https://pubchem.ncbi.nlm.nih.gov/compound/7929, accessed 16 Sep 2020

NCBI (National Center for Biotechnology Information) (2020 b) o-Xylene. PubChem compound summary for CID 7237. https://pubchem.ncbi.nlm.nih.gov/compound/7237, accessed 16 Sep 2020

NCBI (National Center for Biotechnology Information) (2020 c) p-Xylene. PubChem compound summary for CID 7809. https://pubchem.ncbi.nlm.nih.gov/compound/7809, accessed 16 Sep 2020

Peragallo J, Biousse V, Newman NJ (2013) Ocular manifestations of drug and alcohol abuse. Curr Opin Ophthalmol 24(6): 566–573. https://doi.org/10.1097/ICU.0b013e3283654db2

Ramakrishnan A, Lupo PJ, Agopian AJ, Linder SH, Stock TH, Langlois PH, Craft E (2013) Evaluating the effects of maternal exposure to benzene, toluene, ethyl benzene, and xylene on oral clefts among offspring in Texas: 1999-2008. Birth Defects Res A Clin Mol Teratol 97(8): 532–537. https://doi.org/10.1002/bdra.23139

Rosen MB, Crofton KM, Chernoff N (1986) Postnatal evaluation of prenatal exposure to p-xylene in the rat. Toxicol Lett 34(2–3): 223–229. https://doi.org/10.1016/0378-4274(86)90214-6

Rozas G, Guerra MJ, Labandeira-García JL (1997) An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain Res Brain Res Protoc 2(1): 75–84. https://doi.org/10.1016/s1385-299x(97)00034-2

Saillenfait AM, Gallissot F, Morel G, Bonnet P (2003) Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. Food Chem Toxicol 41(3): 415–429. https://doi.org/10.1016/s0278-6915(02)00231-4

Taskinen H, Kyyrönen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm ML (1994) Laboratory work and pregnancy outcome. J Occup Med 36(3): 311–319. https://doi.org/10.1097/00043764-199403000-00008

Ungváry G, Tátrai E (1985) On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. Arch Toxicol Suppl 8: 425–430. https://doi.org/10.1007/978-3-642-69928-3_95

Ungváry G, Tátrai E, Hudák A, Barcza G, Lõrincz M (1980) Studies on the embryotoxic effects of ortho-, meta- and para-xylene. Toxicology 18(1): 61–74. https://doi.org/10.1016/0300-483x(80)90038-4

Ungváry G, Varga B, Horváth E, Tátrai E, Folly G (1981) Study on the role of maternal sex steroid production and metabolism in the embryotoxicity of para-xylene. Toxicology 19(3): 263–268. https://doi.org/10.1016/0300-483x(81)90136-0

US EPA (US Environmental Protection Agency) (2003) Toxicological review of xylenes. EPA/635/R03/001. Washington, DC: US EPA. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0270tr.pdf, accessed 24 Jun 2020

Xiao G, Pan C, Cai Y, Lin H, Fu Z (2001) Effect of benzene, toluene, xylene on the semen quality and the function of accessory gonad of exposed workers. Ind Health 39(2): 206–210. https://doi.org/10.2486/indhealth.39.206