Xylene

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over xyleen
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 2458/AvdB/tvdk/543-C5
Bijlagen : 1
Datum : 20 december 2001

Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve lijst van voor de voortplanting vergiftige stoffen. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1, 2 of 3 wat betreft effecten op de voortplanting en stoffen die schadelijk kunnen zijn voor het nageslacht via de borstvoeding. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In dit kader bied ik u hierbij een advies aan over xyleen. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volkgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

prof. dr JA Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 75 20
email: GR@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23

Xylene

Evaluation of the effects on reproduction, recommendation for classification

Committee for Compounds toxic to reproduction,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

No. 2001/10OSH, The Hague, 20 December 2001

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

Preferred citation:
Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Xylene; Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2000; publication no. 2000/10OSH.

ISBN: 90-5549-405-4

Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie xyleen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte gegevens beschikbaar zijn. Zij adviseert daarom om xyleen niet te classificeren.
- Voor effecten op de ontwikkeling adviseert de commissie xyleen in categorie 3 (*stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens*) te classificeren en met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om xyleen niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed xylene.

The committee's recommendations are

- For effects on fertility, the committee recommends no classification of xylene due to a lack of appropriate data.
- For developmental toxicity, the committee recommends to classify xylene in category 3 (*substances which cause concern for humans owing to possible developmental effects*) and to label xylene with R63 (*possible risk of harm to the unborn child*).
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data xylene should not be labelled.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of xylene by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by dr ir APM Wolterbeek and ir DH Waalkens-Berendsen, of the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies

concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Union listed in Annex C.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 2001, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).

- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterization and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration is above an exposure limit for the general population, eg the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 2000. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

Animal data are described in the text and summarized in Annex D.

1.6 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- Lack of appropriate data preclude assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasizes that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter **2**

Xylene

2.1 Introduction

Name	:	o-, m-, p-xylene
CAS-no	:	1330-20-7
Examples of use	:	Mixed in gasoline, used as a solvent, for the production of phthalic and terephthalic acids and some of their derivatives, as paint, for printing and in the shoe industry
Mol weight	:	106.17
Chem formula	:	C_8H_{10}
density	:	0.88
Conversion factor	:	1 ppm = 4.35 mg/m^3 at 760 mm Hg and 20 ^{o}C 1 mg/m^3 = 0.23 ppm

2.2 Human studies

Fertility

Taskinen *et al.* (Tas89) conducted a nested case-control study of 120 cases with spontaneous abortion and 251 controls on the basis of a file of 6000 Finnish male workers who had been biologically monitored for exposure to six organic solvents (styrene, xylene, toluene, tetrachloroethylene, trichloroethylene and

1,1,1-trichloroethane) at the Finnish Institute of Occupational Health during 1965-1983. Information about their marriages and their wives' pregnancies and spontaneous abortions were obtained from national registries; data on paternal occupational exposure to solvents were collected by means of a questionnaire sent to workers and covered the period of spermatogenesis. The incidence of spontaneous abortions among wives of men occupationally exposed to organic solvents was statistically significantly increased (cases 103, referents 182; adjusted odds ratio 2.3 (95% CI 1.1-5.0)). The incidence of spontaneous abortions among wives of men frequently exposed to xylene or exposed to high concentrations of xylene was slightly, but not statistically significantly, increased (cases n=19, referents n=29; adjusted odds ratio 1.6 (95% CI 0.8-3.2)).

Sallmén *et al.* (Sal95) performed a retrospective time to pregnancy study among women biologically monitored for exposure to six organic solvents (styrene, xylene, toluene, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane) at the Finnish Institute of Occupational Health during 1965-1983 (n=3265). In this study 197 women participated. More than half of the subjects (105) were exposed to organic solvents during their time to pregnancy. Nearly a quarter were highly exposed (handling solvents daily or 1-4 days per week supported by individual exposure measurements). Exposure to organic solvents was significantly related with reduced fecundity after adjustment for confounding factors (incidence density ratio of clinical pregnancies was 0.69 (95% CI 0.48-0.99) and 0.41 (95% CI 0.27-0.62) for low and high exposure, respectively). The incidence density ratios for workers exposed to xylene were 1.41 (95% CI 0.91-2.20) (low exposure, n=31) and 0.93 (95% CI 0.47-1.84) (high exposure, n=10).

In a prospective study of Lemasters *et al.* (Lem99) sperm production, structure, and function were evaluated in a total of 50 exposed (divided into four groups: sheet metal workers (n=6), painters (n=6), jet fuels workers (n=15), flight line crews (n=23)) and 8 control men working on aircraft maintenance at an Air Force installation before and at 15 and 30 weeks after exposures had begun. Industrial hygiene sampling and expired breath samples were collected for analysis of, among other components, xylene and toluene. Mean exposure levels were low (< 6 ppm (as a mixture) = 26.1 mg/m^3). For most sperm measures, mean values remained in the normal range throughout the 30-week exposure period. Men in the paint shop group, with a higher exposure to solvents, had a significant decline in motility of 19.5% at 30 weeks, however there was no significant association between internal dose and spermatogenic changes.

In a recent study of De Celis *et al.* (Dec00), the effect of occupational exposure of men to hydrocarbons on the quality of their sperm was determined in 48 workers who were exposed to hydrocarbons for 2-24 years and in 42 unexposed workers at a rubber factory located in Mexico City. The environmental concentrations of the hydrocarbons were determined by continuous monitoring of all factory areas during the workday; the measured concentrations were as follows: ethylbenzene 220.7-234 mg/m^3, benzene

31.9-47.8 mg/m^3, toluene 189.7-212.5 mg/m^3 and xylene 47-56.4 mg/m^3. The incidence of abnormal characteristics found in the semen of exposed men was higher than in the semen of unexposed workers, including alterations in viscosity (P<0.001, slight correlation between incidence of abnormal viscosity and duration of exposure), liquefaction capacity (P=0.002, slight inverse correlation between the incidence of increased liquefaction and duration of exposure), sperm count (P<0.001, lower sperm count was associated with longer duration of exposure [r=0.52]), sperm motility (P<0.001, inverse correlation between motility and duration of exposure [r=0.57]) and the proportion of sperm with normal morphology (P<0.001, abnormalities were more common in workers who were exposed for a longer period [r=0.51]).

Development

Holmberg (Hol79) and Holmberg and Nurminen (Hol80) identified 132 cases of children with congenital central nervous system defects in two-year data of the Finnish Register of Congenital Malformations (articles described the same population). One hundred twenty of them and their referents were included in these studies. Fourteen (Hol79) and 12 (Hol80) case mothers and 3 referent mothers reported exposure to (various) organic solvents (amongst others styrene, acetone, white spirit, toluene, xylene) during pregnancy. Statistical analysis of these data showed a significant association between organic solvent exposure during pregnancy and the incidence of congenital central nervous system defects.

In a case-referent study of Kurppa *et al.* (Kur83) the relationship between exposure to organic solvents and the incidence of congenital malformations was investigated. Data were derived from the Finnish Register of Congenital Malformations. Initial two-year data showed an association between maternal exposure to organic solvents and defects of the central nervous system among children born to these mothers (14 case and 3 referent mothers had been exposed to solvents in early pregnancy; most probably the same population as described in the articles of Holmberg (Hol79 and Hol80)). However, for the following three-year period this association was no longer detectable (respective distribution: 6 cases and 6 referents).

In a case-control study of Taskinen *et al.* (Tas86) 44 case mothers (having a spontaneous abortion) working in a pharmaceutical factory were each age-matched to 3 control mothers (having given birth) working in the pharmaceutical factory. Although the odds ratio for spontaneous abortions were increased after exposure to single solvents, no statistically significant effects on the incidence of spontaneous abortion were observed (odds ratio of spontaneous abortion for xylene exposure was 2.0 (95% CI 0.4-10.6)). The frequency of exposure to solvents affected the incidence of spontaneous abortion but these data were not presented for xylene. Furthermore, the

odds ratio for spontaneous abortion was greater among women exposed to a mixture of > 4 solvents (cases n=8, control n=9; odds ratio 3.5 (95% CI 1.0-12.4)) than among women exposed to a mixture of < 3 solvents (cases n=4, controls n=17; odds ratio 0.8 (95% CI 0.3-2.6)).

In the study of Taskinen *et al.* (Tas89, already described in the section about fertility) there was no significant association between paternal exposure to xylene and the incidence of congenital malformations (odds ratio 1.6 (95% CI 0.4-5.7)). Furthermore, no significant effect on the incidence of spontaneous abortions was observed for maternal exposure to organic solvents (cases n=11, referents n=18; adjusted odds ratio 1.2 (95% CI 0.5-2.7)).

Lindbohm *et al.* (Lin90) studied the association between medically diagnosed spontaneous abortions and maternal occupational exposure to organic solvents. The final population for the analysis was restricted to the matched case-control sets who confirmed their pregnancy and reported in detail their occupational exposures during early pregnancy (73 cases of spontaneous abortion and 167 controls). The incidence of spontaneous abortions was increased among the women exposed to organic solvents (58%) compared to controls (42%); odds ratio 2.2 (95% CI 1.2-4.1). The odds ratio of spontaneous abortion for xylene exposure was 1.3 (95% CI 0.4-4.5) (5 cases and 7 controls).

Spontaneous abortions, congenital malformations and birth weight of children of women working in laboratories were examined in a retrospective case-referent study of Taskinen *et al.* (Tas94). Frequent exposure to xylene (at least 3 days per week) was statistically significantly associated with spontaneous abortion (cases n=16, referents n=12, odds ratio 3.1 (95% CI 1.3-7.5)). No such association was observed for less frequent exposure to xylene (1 or 2 days a week; cases 19, referents 27, odds ratio 1.3 (95% CI 0.7-2.5)). No association with birth weights or incidence of congenital malformations was observed.

In a retrospective study among women who had been exposed to solvents, a comparison was made between those who have had a spontaneous abortion at less than 20 weeks of gestation and those who produced normal live births in order to identify a correlation between solvent exposure and risk of spontaneous abortion (Win91). No such association was found when general solvent exposure was examined (89 cases and 160 control; crude odds ratio 1.2 (95% CI 0.87-1.60)) nor when xylene exposure was examined (9 cases and 12 controls; crude odds ratio: 1.6 (95% CI 0.66-3.8)).

Lactation

Fisher *et al.* (Fis97) studied the human blood/air and milk/air partition coefficient (PC) in human blood and human milk samples. The objective of this study was to evaluate the

potential chemical exposure of a nursing infant by ingestion of contaminated milk from a mother who was occupationally exposed to vapours. To estimate infant exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on an 8-hour exposure period of the mother to a constant vapour concentration equal to the threshold limit value for xylene (100 ppm = 435 mg/m^3). The experimentally determined blood/air and milk/air PC values were used in the PB-PK lactation model. The predicted amount of xylene ingested by a nursing infant over a 24-hour period was 6.59 mg in 0.92 l (7.16 mg/l).

Based on a MAC value for xylene of 210 mg/m^3, a maximal permissible level of about 175 mg/l breast milk, can be calculated for xylene (see Annex E).

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the fertility and developmental toxicity studies with xylene in experimental animals.

Fertility studies

In a study by Ungváry *et al.* (Ung81), CFY rats were exposed to p-xylene (3000 mg/m^3) by inhalation on GD 10 or GD 9-10. Rats were sacrificed on GD 11. Foetal weights (see section about developmental toxicity) and ovarian secretion of progesterone and 17ß-oestradiol and the concentrations of ovarian secretions (progesterone and 17ß-oestradiol) were measured in uterine and femoral blood. Maternal toxicity was not presented. No effect was observed on ovarian secretion of hormones but the levels of these hormones in the uterine and femoral blood were decreased after 48 h of exposure suggesting an effect of p-xylene on the metabolism of these hormones by the hepatic monooxygenase system.

In a study by Biodynamics (Bio83), male and female Sprague Dawley rats were exposed by inhalation to a mixture of xylenes at concentrations of 0, 60, 250 or 500 ppm (0, 261, 1088 or 2175 mg/m^3) for 6 hours/day during 131 days before mating and 20 days mating period. The mated females were also exposed during gestation from GD 1 to 20 and during days 5 to 20 of the lactation period. Additionally, exposed males from the highest dose group were mated with unexposed females and vice versa. Approximately one third of the pregnant females exposed to 0 or 2175 mg/m^3 were used in a teratogenicity study (see section about developmental toxicity) and the remaining animals delivered their young. There were no mortalities in the treated groups during the study. Body weight gain was significantly higher during the mating period in females exposed to 261 or 1088 mg/m^3. There were no treatment-related effects on

mating, fertility, pregnancy indices, mean duration of gestation, mean litter size or mean pup weight.

Nylen *et al.* (Nyl89) found no alterations in testes, accessory glands or circulating male hormone levels after inhalatory exposure of male Sprague Dawley rats to 1000 ppm (4350 mg/m^3) mixed xylenes for 61 days, 18 hours/day, 7 days a week. No dead animals nor any body weight changes were observed. Furthermore, all rats exposed to xylene were fertile.

Wistar rats were exposed to xylenes twice a day for 7 days in an inhalation chamber (concentration level is not described; cotton was soaked in 20 ml of xylene (composition not specified)). Animals were kept in the inhalation chamber until anaesthesia was achieved (after about 10 min). Rats were sacrificed at day 7. Exposure to xylenes reduced the weight of the testes and accessory reproductive organs. Furthermore, a decreased plasma testosterone level, acid phosphatase activity in the prostate and a decreased number of spermatozoa in the epididymides were observed in this study. On the 7th day of treatment, body weights were decreased (Yam93).

Male Sprague Dawley rats were injected once intraperitoneally with 0, 0.5 or 1.5 ml (0, 0.4 or 1.3 g) o-xylene/kg body weight (Was83). The animals were housed at two temperatures 20-24 ^{0}C and 24-30 ^{0}C. Five weeks after treatment, the animals were sacrificed and an analysis of sperm was conducted. No significant increase in abnormal sperm was observed in the xylene-treated animals housed at 20-24 ºC. Rats housed at temperatures between 24-30 ºC showed a significant increase in abnormal sperm after intraperitoneal injection with 0.5 ml o-xylene/kg body weight. General toxicity was not described in this study.

Developmental toxicity

CFY rats were exposed by inhalation to 0 and 1000 mg/m^3 xylene (mixture) for 24 hour/day from gestation day (GD) 9-14 (Hud78). No maternal toxicity was observed. At foetal skeletal examination of the xylene exposed group, an increase in the frequency of fused sternebrae and extra ribs was observed. However, it should be noted that the number of foetuses per litter in this group was 14.30 versus 11.25 in the controls; the observed developmental effects could be caused by the higher number of foetuses in the xylene treated group.

In a study of Ungváry *et al.* (Ung80), CFY rats were exposed by inhalation to 0, 150, 1500 or 3000 mg/m^3 of either o-, m- or p-xylene on GD 7-14 for 24 hours/day. On the 21st day of pregnancy, animals were sacrificed. Statistically significantly increased liver weights of the dams were observed in all o-xylene treated groups. Although during the exposure period maternal weight gain was dose dependently decreased, on GD 21 it was not affected except for the body weight gain of the rats of the high dose group of m-xylene which was decreased. In this group also mortality of the dams (4 out of 30 died) was observed. From GD 7-14, food consumption was decreased in the o-, m- and p-xylene-treated females in a dose-dependent way. Foetal loss was increased in the high dose group of p-xylene and foetal weight was decreased in the high dose groups of all isomers and in the mid dose group of o-xylene. Skeletal retardation was observed in the high dose group of o-xylene and in all dose groups of p-xylene. Extra ribs were observed in the high dose group of m- and p-xylene.

In another study of Ungváry *et al.* (Ung81), CFY rats were exposed to p-xylene (3000 mg/m^3) by inhalation on GD 10 or GD 9-10. Rats were sacrificed on GD 11 and foetal weights and ovarian secretion of progesterone and 17ß-oestradiol and the concentrations of ovarian secretions (progesterone and 17ß-oestradiol) were measured in uterine and femoral blood (see section about fertility). Maternal toxicity was not presented. Foetal weight was decreased after a 48 hour exposure period.

In a Biodynamics teratogenicity study (Bio83), there were no effects on the number of implantation sites, live foetuses per implant or sex distribution (for a description of the study see section about fertility). There was no effect on the incidence of foetuses with delayed development or malformations in the groups exposed to xylene. During the lactation period there were no effects on the pups. On postnatal (PN) day 49, in the group where both parents had been exposed to 500 ppm (2175 mg/m^3), mean pup weight (both male and female) was significantly lower than controls but there was no effect on pup gonads weights.

In a study of Mirkova *et al.* (Mir83), pregnant Wistar rats were exposed to xylene (0, 10, 50 and 500 mg/m^3, industrial mixture of isomers) by inhalation for 6 hours/day, 5 days/week from GD 1-21. On GD 21, some of the animals were sacrificed for foetal examinations and the remaining dams were allowed to litter. Maternal toxicity as well as composition of the xylene mixture were not presented. A significant increase of post-implantation loss and a significant decrease of mean foetal weight were observed in the 50 and 500 mg/m^3 groups. Furthermore, a significant increase in the incidence of haemorrhages in the thoracic and abdominal cavities was observed in these groups (39%, 46% and 53% respectively) although the incidence in the control group was also very high (31%). Foetal examination revealed a statistically significant increased incidence of anomalies of the internal organs (500 mg/m^3 group) and of foetuses showing a delayed skeletal development (50 and 500 mg/m^3). However, these data

were not presented in detail. In the groups which delivered their young, there was no effect on postnatal mortality but pup body weights were statistically significantly decreased at 50 and 500 mg/m^3 on PN days 7 and 21 and not statistically significantly on PN days 4 and 45. Furthermore, the activities of several metabolic enzymes were altered in pups of the two highest dosing groups and the pups of the 500 mg/m^3 group showed altered results of behavioural tests.

Ungváry & Tátrai (Ung85) studied the effects of 0, 250, 1900 or 3400 mg/m^3 xylene (mixture of isomers) in CFY rats after inhalation for 24 hours/day from GD 7-15. Maternal toxic effects were moderate and dose dependent (not specified). In the highest dose group 1 dam died. In all dose groups, the incidence of foetuses with skeletal retardation was increased, however this effect was not dose related. Furthermore, the incidences of resorptions, foetuses with extra ribs and weight retarded foetuses were increased in the highest dose group.

Furthermore, CFLP mice were exposed by inhalation from gestation days 6-15 to 0 and 500 mg/m^3 o-, m- or p-xylene and 500 and 1000 mg/m^3 xylene (mixture of isomers) during 3 x 4 hours/day (Ung85). Maternal toxicity was not presented. At 500 mg/m^3 o-, m- and p-xylene and at 1000 mg/m^3 of the mixture of isomers, the incidences of weight and skeletal retarded foetuses was increased.

Furthermore, Ungvary & Tatrai (Ung85) exposed New Zealand White rabbits by inhalation from GD 7-20 to 0, 500 or 1000 mg/m^3 p-xylene, 500 mg/m^3 o- or m- xylene, 500 or 1000 mg/m^3 mixed xylene isomers for 24 hours/day (n=8-10). In the groups exposed to 500 mg/m^3 o- or p-xylene no effects were observed. After exposure to 500 mg/m^3 m-xylene, an increased number of resorptions was noted. However, in the 1000 mg/m^3 p-xylene group, overt maternal toxicity was observed, 1 dam died and 3 dams aborted; 4 dams showed total resorptions or death of foetuses at Caesarian section. In the 500 mg/m^3 mixed xylene group, decreased fetal (female) weights were observed. In the 1000 mg/m^3 xylene (mixture) 3 dams died and 6 dams aborted; 1 dam showed total resorptions or death of foetuses at Caesarian section. The relative liver weight of this group was increased. The percentage of malformations was comparable in all groups.

Sprague Dawley rats were exposed by inhalation to 0, 3500 or 7000 mg/m^3 p-xylene for 6 hours per day on GD 7-16 and the offspring were evaluated for growth, viability and neurobehavioural development (Ros86). The high dose level reduced maternal weight gain during the exposure period, but growth, viability, locomotor activity and acoustic startle response of the offspring were not affected.

Mated female Wistar rats were exposed by inhalation to technical xylene (mixture), 0 or 200 ppm (870 mg/m^3) during 6 hours per day on GD 4 to 20 (Has93). There were no signs of maternal toxicity. In the prenatal developmental toxicity study, no exposure-related differences were found except for delayed ossification of os maxillae. In the postnatal part of the study, pups of the xylene exposed dams had a higher body

weight, showed a delayed ear folding and eye opening and an impaired performance of a motor ability test (rotorod test).

In another study, Hass *et al.* (Has95) studied the postnatal effects in mated female Wistar rats that inhaled a mixture of technical xylene (0 and 500 ppm, 2175 mg/m^3) for 6 hours per day on GD 7-20. In the exposed offspring, a delay in the righting reflex, a lower absolute brain weight, and impaired performance in behavioural tests for motor abilities (Rotarod) and for learning and memory (Morris water maze) were found. Generally, the effects were most marked in the female offspring. A slight, negligible effect on body weight was observed. No maternal toxicity was observed.

In a comparable study of Hass *et al.* (Has97), pregnant Wistar rats were exposed for 6 hour per day on GD 7-20 of pregnancy to 0 or 500 ppm (2125 mg/m^3) technical xylene mixture. After weaning, one female pup per litter was selected for investigations of learning and memory abilities using a Morris water maze. Impaired performances were observed in exposed offspring at an age of 16, 28 and 55 weeks, although the difference at 55 weeks was not statistically significant. Maternal toxicity data were not presented.

In a study of Kükner *et al.* (Kük97) pregnant Wistar rats were exposed by inhalation to xylene (11284 mg/m^3) from GD 6 to 21 for 8 hours a day. Maternal body weights were not affected. No external anomalies were observed in any of the pups and there were no macroscopic defects in their organs. (Electron) microscopy revealed xylene induced structural defects in the liver of pregnant and non-pregnant dams and in the livers of the pups of dams exposed to xylene. Furthermore, activities of several hepatic enzymes were altered in the livers of the dams.

Lactation

No publications concerning concentrations of xylene in milk or effects of exposure during lactation were found.

2.4 Conclusion

Occupational exposure to mixtures of organic solvents, including xylenes, has been shown to increase the incidence of spontaneous abortions among wives of exposed men (Tas89), to decrease fecundity of exposed women (Sal95) and to increase the incidence of abnormal characteristics of sperm of exposed men (Lem99; DeC00). In the studies of Taskinen *et al.* and of Sallmén *et al.* no effects of exposure to xylene (no other exposures) on abortions and fecundity were observed. In the studies of Lemasters *et al.* and of De Celis *et al.* only the effects of exposure to a mixture of solvents on sperm parameters were studied.

Yamada (Yam93) reported effects in male rats on testis and accessory reproductive organs and testosterone level after inhalatory exposure to xylene. Animals were exposed to xylene twice per day for 7 days until anaesthesia occurred (the concentration of xylene was not measured but most probably, the actual concentration of xylene in the exposure chamber was very high); In addition, in the exposed group effects on body weight were observed. In other studies no effect on reproductive organs, sperm number or fertility were observed (Bio83, Was83, Nyl89). However, in the Biodynamics study (Bio83), no maternal toxic dose was reached.

In conclusion, due to a lack of appropriate data, the committee recommends not to classify xylene for effects on fertility.

Holmberg (Hol79 and Hol80) and Kurppa *et al.* (Kur83) reported developmental effects on the central nervous system after exposure to organic solvents during pregnancy but this association was not found in a follow-up study. In two human studies of Taskinen *et al.*, no significant effects of occupational exposure of women to xylene (Tas86) or to organic solvents (Tas89) on the incidence of spontaneous abortion were observed. Furthermore, in the 1989 study of Taskinen *et al.* there was no association between paternal exposure to xylene and the incidence of congenital malformations. However, Taskinen *et al.* described in 1994 that maternal exposure to xylene for a minimum of at least three days per week resulted in a (statistically significant) increased incidence of spontaneous abortion (Tas94). Exposure to xylene did not result in an lower birth weights or congenital malformations in new-borns. Lindbohm *et al.* (Lin90) described that the incidence of spontaneous abortions was increased among women exposed to organic solvents but not when exposed to xylene alone whereas Windham *et al.* (Win91) did not find any association between exposure to organic solvents or xylene and the incidence of spontaneous abortion.

In the study of Hudak *et al.* (Hud78) the observed effects on sternebrae and ribs in CFY rats treated with 1000 mg/m^3 xylene might be due to the higher number of foetuses rather than to xylene treatment. In the study of Ungváry *et al.* (Ung80) the effects of o-, m- or p-xylene in rats on foetal loss, foetal weight, skeletal retardation and the incidence of extra ribs were observed at dose levels which induced maternally toxic effects. In the study of Ungváry *et al.* (Ung81), showing foetal weight loss, maternal toxicity was not presented but the very high dose level used most probably induced toxic effects. Although in the Biodynamics study (Bio83) no effect of xylene was observed on the incidence of foetuses showing delayed development or malformations, mean weight of the pups (in the group in which both parents were exposed to 2175 mg/m^3 xylene) on PN day 49 was significantly decreased. However, no maternally toxic doses were reached. The results of the study of Mirkova *et al.* (Mir83) are questionable since the incidence of haemorrhages observed in the thoracic and abdominal cavities of all

groups, including the control group, is very high indicating a poor condition of the rats. The developmental effects of xylene in rats, mice and rabbits observed by Ungváry and Tátrai (Ung85) appeared at (likely) maternal dose levels. In the study of Rosen *et al.* (Ros86) no developmental effects are observed notwithstanding the high, maternally toxic, dose levels of xylene. In a series of studies of Hass *et al.* (Has93, Has95, Has97) several neuro-developmental effects were observed in the offspring of rats which were exposed to relative low, not maternally toxic, dose levels of xylene (870, 2175 mg/m^3) from GD 4 or 7 to 20.

In view of the animal data concerning developmental toxicity, malformations, decreased foetal weight, skeletal retardation, effects on the developing nervous system in relation with the maternal toxicity, the committee recommends to classify xylene (isomers) in category 3 (substances which cause concern for humans owing to possible developmental toxic effects) and to label the compound with R 63 (possible risk of harm to the unborn child).

Little or no data concerning the presence of xylene in milk and its effects on development in men and animals are available.

From the study of Fisher *et al.* (a pharmacokinetic lactation model), an amount of 7.2 mg xylene/l breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) xylene concentration in human breast milk can only be used as an indication for the possible amount of the compound in breast milk, because the model is not yet sufficiently validated. The committee concludes that the predicted exposure level is no reason for labelling. No experimental data are available about the concentration of xylene in human breast milk and about the possible effects during lactation.

In conclusion, the committee proposes not to label xylene for effects during lactation because of lack of appropriate data.

Proposed classification for fertility

Lack of appropriate data precludes assessment of xylene for effects on fertility.

Proposed classification for developmental toxicity

Category 3, R 63

Proposed labelling for effect during lactation

Lack of appropriate data precludes assessment of xylene for effects during lactation.

Additional consideration

The committee would like to emphasize that several human studies considered here in view of xylene exposure give reason for concern with respect to effects on fertility and development. However, it is not clear in these studies whether exposure involved pure xylene or a mixture of solvents containing xylene. Therefore, the EU Classification and Labelling guideline does not warrant a classification of xylene on the basis of these human studies. However, the committee emphasizes that there is clearly cause for concern for effects on fertility and development after exposure to mixtures of solvents containing xylene.

References

Bio83 Biodynamics. Parental and fetal reproduction inhalation toxicity study in rats with mixed xylenes. Project no. 80-2520. 1983

DeC00 De Celis R, Feria-Velasco A, González-Unzaga M, Torres-Calleja J, Pedrón-Nuevo N. Semen quality of workers occupationally exposed to hydrocarbons. Fertility and Sterility 2000; 73: 221-228.

Fis97 Fisher J, Mahle D, Bankston L, Greene R, Gearhart J. Lactational transfer of volatile chemicals in breast milk. Am. J. Ind. Hyg. Assoc. J. 1997; 58: 425-431.

Has93 Hass U, Jakobsen BM. Prenatal toxicity of xylene inhalation in the rat: A teratogenicity and postnatal study. Pharmacology & Toxicology 1993; 73: 20-23.

Has95 Hass U, Lund SP, Simonson L, Fries AS. Effects of prenatal exposure to xylene on postnatal development and behaviour in rats. Neurotox. Teratol. 1995; 17: 341-349.

Has97 Hass U, Lund SP, Simonsen L Long-lasting neurobehaviourial effects of prenatal exposure to xylene in rats. Neurotoxicology 1997; 18: 547-552.

Hol79 Holmberg, PC. Central-nervous-system defects in children born to mothers exposed to organic solvents during pregnancy. Lancet ii; 1979: 177-179.

Hol80 Holmberg PC, Nurminen M. Congenital defects of the central nervous system and occupational factors during pregnancy. A case-referent study. Am. J. Ind. Med. 1980; 1: 167-176.

Hud78 Hudak A, Ungvary G. Embryotoxic effects of benzene and its methyl derivates: Toluene, xylene. Toxicology 1978; 11: 55-63.

Kük97 Kükner A, Canpolat L, Ozan E, Gökçimen A, Ozan S, Do rul M. The effect of xylene inhalation on the rat liver. Acta Phys. Hung. 1997/1998; 85: 231-241.

Kur83 Kurppa K, Holmberg PC, Hernberg S, Rantala K, Riala R, Nurminen T. Screening for occupational exposures and congenital malformations. Preliminary results from a nationwide case-referent study. Scand. J. Work Environ. Health 1983; 9: 89-93.

Lem99 Lemasters GK, Olsen DM, Yiin JH, Lockey JE, Shukla R, Selevan SG, Schrader SM, Toth GP, Evenson DP, Huszar GB. Male reproductive effects of solvents and fuel exposure during aircraft maintenance. Reproductive Toxicol. 1999; 13: 155-166.

Lin90 Lindbohm M-L, Taskinen H, Sallmén M, Hemminki K. Spontaneous abortions among women exposed to organic solvents. Am. J. Ind. Med. 1990; 17: 449-463.

Mir83 Mirkova E, Zaikov Chr, Antov G, Mikhailova A, Khinkova L, Benchev IV. Prenatal toxicity of xylene. J. Hyg. Epidemiol. Microbiol. Microbiol. 1983; 27: 337-343.

Nyl89 Nylen P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson A-C, Kronevi T, Kvist U, Sjöstrand NO, Höglund G, Olson L. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch. Toxicol. 1989: 63: 296-307.

Ros86 Rosen MB, Crofton KM, Chernoff N. Postnatal evaluation of prenatal exposure to p-xylene in the rat. Toxicol. Letters; 1986; 34: 223-229.

Sal95 Sallmén M, Lindbohm M-L, Kyyrönen P, Nykyri E, Antilla A, Taskinen H, Hemminki K. Reduced fertility among women exposed to organic solvents. Am. J. Ind. Med. 1995; 27: 699-713.

Tas86 Taskinen H, Lindbohm M-L, Hemminki K. Spontaneous abortions among women working in the pharmaceutical industry. Br. J. Ind. Med. 1986; 43: 199-205.

Tas89 Taskinen H, Antilla A, Lindbohm M-L, Sallmén M, Hemminki K. Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents. Scand. J. Work Environ. Health 1989; 15: 345-352.

Tas94 Taskinen H, Kyyrönen P, Hemminki K, Hoikkala M, Lajunen K, Lindbohm M-L. Laboratory work and pregnancy outcome. J Occupational Medicine 1994; 36: 311-319.

Tox95 Niesink RJM, de Vries J, Hollinger MA, eds, Toxicology, Principles and Applications, Boca Raton: CRC Press, 1995:385.

Ung80 Ungvary G, Tatrai E, Hudak A, Barcza G, Lörincz M. Studies on the embryotoxic effects of ortho-, meta- and para-xylene. Toxicology 1980;18: 61-74.

Ung81 Ungváry G, Varga B, Horváth E, Tátrai E, Folly G. Study on the role of maternal sex steroid production and metabolism in the embryotoxicity of para-xylene. Toxicology 1981; 19: 263-268.

Ung85 Ungvary G, Tatrai E. On the embryotoxic effects of benzene and its alkyl derivates in mice, rats and rabbits. Arch. Toxicol. 1985; suppl. 8: 425-430.

Was83 Washington WJ, Murthy RC, Doye A, Eugene K, Brown D, Bradley I. Induction of morphologically abnormal sperm in rats exposed to o-xylene. Archives of Andrology 1983; 11: 233-237.

Win91 Windham GC, Shusterman D, Swan SH, Fenster L, Eskenazi B. Exposure to organic solvents and adverse pregnancy outcome. Am. J. Ind. Med. 1991; 20: 295-301.

Yam93 Yamada K. Influence of lacquer thinner and some organic solvents on reproductive and accessory reproductive organs in the male rat. Biol. Pharm. Bull.1993; 16: 425-427.

Literature consulted but not cited

Age95 Agency for toxic substances and disease registry (ATSDR). Toxicological Profile for xylene 1995.

Axe84 Axelsson G, Lütz C, Rylander R. Exposure to solvents and outcome of pregnancy in university laboratory employees. Br. J. Ind. Med. 1984; 41: 305-312.

Bar82 Barlow SM, FM Sullivan. Xylene. In: Reproductive hazards of industrial chemicals. An evaluation of animal and human data. (ed: S.M. Barlow) Acad. Press, London, 1982: 592-599.

Bel92 Bell GM, Shillaker RO, Padgham MDJ, Standring P. Xylenes. Health & Safety Executive (HSE) Toxicity Review 26, 1992.

Bro91 Brown-Woodman PDC, Webster WS, Picker K, Ritchie HE. Embryotoxicity of xylene and toluene: an in vitro study. Ind. Health 1991; 29: 139-152.

Bro94 Brown-Woodman PDC, Webster WS, Picker K, Huq F. In vitro assessment of individual and interactive effects of aromatic hydrocarbons on embryonic development of the rat. Reproductive Toxicol. 1994; 8: 121-135.

DEC91 Dutch expert committee for occupational standards. Health-based recommended occupational exposure limit for xylene. 1991.

Gha86 Ghantous H, Danielsson BRG. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol. Res. Pregnancy 1986; 7: 98-105.

Has87 Hass U, Jakobsen BM. Prenatal toxicity of xylene inhalation in the rat: teratogen study and behavioural study. Teratol. 1987; 36: 25A-26A (abstract).

Has92 Hass U, Lund SP. Deficits on a spatial navigation task following prenatal exposure to xylene. Teratol. 1992; 46: 18A (abstract).

Has95 Hass U, Lund SP, Simonsen L. Long-lasting neurobehavioural effects of prenatal exposure to xylene in rats. Neurotoxicol 1995; 16: 761 (abstract).

Hoo85 Hood RD, Ottley MS. Developmental effects associated with exposure to xylene: A review. Drug and Chemical Toxicology 1985; 8: 281-297.

IAR89 IARC monographs on the evaluation of carcinogenic risks to humans. Some organic solvents, resin monomers and related compounds, pigments and occupational exposures in paint manufacture and painting. Volume 47: 1989.

IAR99 IARC monographs on the evaluation of carcinogenic risks to humans. Re-evaluation ofsome organic chemicals, hydrazine and hydrogen peroxide. Volume 71: 1999

Joh86 Johnson EM, Gabel BEG, Christian MS, Sica E. The developmental toxicity of xylene and xylene isomers in the Hydra assay. Toxicol. Appl. Pharmacol. 1986; 82: 323-328.

Krä99 Krämer A, Linnnert M, Wrbitzky R. Angerer J. Occupational chronic exposure to organic solvents XVII. Ambient and biological monitoring of workers exposed to xylenes. Int. Arch. Occup. Environ. Health. 1999; 72: 52-55.

Kuc68 Kucera J. Exposure to fat solvents: A possible cause of sacral agenesis in man. J. Pediatr. 1968; 72: 857-859.

Lan94 Langman JM. Xylene: its toxicity, measurement of exposure levels, absorption, metabolism and clearance. Pathology 1994; 26: 301-309.

Mar82 Marks TA, Ledoux TA, Moore JA. Teratogenicity of a commercial xylene mixture in the mouse. J. Toxicol. Environ. Health 1982; 9: 97-105.

Mic65 Michon S. Disturbances of menstruation in woman working in an atmosphere polluted with aromatic hydrocarbons. Pol. Tyg. Lek 1965; 20: 1648-1649 (article in Polish with abstract in English).

Mir79 Mirkova E, Hinkova L, Vassileva L, Bogdanova N. Xylene neurotoxicity in pregnant rats and fetuses. Activ. Nerv. Supp. 1979; 21: 265-268.

Naw80 Nawrot PS, Albro PW, Staples RE. Distribution and excretion of ^{14}C-m-xylene in pregnant mice. Teratology 1980; 21,3:58A (abstract)

Nov79 Novikov KI. Kolodina LN, Lipovskij SM, Badalova LV. Lactation particulars in female workers of the chemical (rubber) industry. Gyg Tr. Prof Zabol 1979; 2: 45-48 (article in Russian).

Ove81 Overman DO. Testing for percutaneous embryotoxicity of laboratory reagents in the hamster. Teratology 1981; 23: 56A

Sch93 Schardein JL. Chemically induced birth defects. 2nd ed., rev. and expanded, Marcel Dekker, Inc., New York, 1993: 751-775.

Shi83 Shigeta S, Aikawa H, Misawa T, Suzuki K. Fetotoxicity of inhaled xylene in mice. Teratology 1983; 28: 22A (abstract)

Sei86 Seidenberg JM, Anderson DG, Becker RA. Validation of an in vivo developmental toxicity screen in the mouse. Terato Carcino Mutagen 1986; 6: 361-374.

Sei87 Seidenberg JM, Becker R A. A summary of the results of 55 chemical screened for developmental toxicity in mice. Teratogenesis, Carcinogenesis and Mutagenesis 1987; 7: 17-28.

She92 Shepard TH. Catalog of teratogenic agents, 7th edition, John Hopkins University Press, Baltimore MD. 1992: 673.

Sul93 Sullivan FM, Watkins WJ, Van Der Venne M Th. Reproductive Toxicity: Xylene. 1993; 1: 410-420.

Syr73 Syrovadko ON, Skornin VF, Pronkova EN, Sorkina MS, Izyumova AS, Gribova IA, Popova AF. Effect of working conditions on the health status and some specific functions of women handling white spirit. Gigiena Truda I Professional Nye Zabolevaniya 1973; 6:5-8 (article in Polish with abstract in English).

Tab86 Tabacova S. Maternal exposure to environmental chemicals. Neurotoxicol 1986; 7: 421-440.

Ung85 Ungváry G. The possible contribution of industrial chemicals (organic chemicals) to the incidence of congenital defects caused by teratogenic drugs and consumer goods - an experimental study. Prog. Clin. Biol. Res. 1985; 160: 295-300.

WHO97 WHO. Xylenes. Environ. Health Criteria 190. 1997.

A The committee

B Comments on the public draft

C Directive (93/21/EEG) of the European Community

D Fertility and developmental toxicity studies

E Calculation safe levels of xylene in (human) breast milk

F Abbreviations

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist, Institute for Risk Assessment Sciences (IRAS), Utrecht
- JN van den Anker
  Professor of pediatrics and Neonatology, Erasmus University, Rotterdam
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- HFP Joosten
  Toxicologist, NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  professor of Clinical Genetics/Teratology, Erasmus University, Rotterdam
- JHJ Copius Peereboom-Stegeman
  Toxicologist, Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist, National Institute of Public Health and the Environment, Bilthoven
- A Stijkel
  Toxicologist, Environmental Awareness Foundation, ‘s-Graveland
- DH Waalkends-Berendsen
  Reproductive toxicologist, TNO Nutrition and Food Research, Zeist
- PJJM Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen

- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by APM Wolterbeek and DH Waalkens-Berendsen, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: M Javanmardi and T van der Klugt.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2001 for public review. The following persons or organisations have commented on the draft document:

- RD Zumwalde
  National Institute for Occupational Safety and Health, USA

Annex **C**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear resuts in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:

T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:

T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:

Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1 *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would

interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri-postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would

lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as 'Toxic to Reproduction', unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or

b on the basis of results of one or two generation studies in animals which indicate the presence of adverse effects on the offspring due to transfer in the milk, and/or

c on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

See next pages.

Table 1 Fertility studies in animals with xylene.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs/ reproduction	remarks
Ungváry *et al*. (1981)	CFY rats (F:n=20)	treament on GD 9 or GD 9-10 sacrifice GD 11	0 or 3000 mg/m^3 p-xylene. 24h/d by inh.	not presented	no effect on ovarian progesterone and oestradiol secretion but concentration of these hormones were decreased in uterine and femoral blood after 48 h exposure	
Bio-dynamics (1983)	Sprague Dawley rats (M:n=10-30; F: n=20-60)	131 d. premating, mating, GD 1-20, PN d 5-20 sacrifice, PN d 21 and 49 (pups)	0,261, 1088 or 2175 mg/m^3 xylene (mix), 6h/d, 7 d/w by inh.	no treatment related effects except for increased bw of females of 261 and 1088 mg/m^3 group during mating	no effects on mating, fertility, pregnancy incidence, mean duration of gestation, mean litter size and mean pup weight.	
Nylon *et al*. (1989)	Sprague Dawley rats (M: n=18)	treatment for 61 d. sacrifice: 2 w, 10m, 14m after end of exposure.	0 or 4350 mg/m^3 xylene, 18 h/d, 7 d/w by inh.	no dead animals nor effects on body weights were observed	no effects on testis, accesory sex glands or hormone levels no effect on fertility	composition xylene not presented
Yamada (1993)	Wistar rats (M: n=5)	twice a d. for 7 d, sacrifice d. 8	rats were exposed by inh until anaesthesia was achieved after about 10 minutes	on the 7th d of treatment decreased body weight	decreased weight of testes, epidymides, vas deferens, seminal vesicles and prostate, decreased levels of plasma testosterone, decreased activity of acid phosphatase in the prostate and decreased number of spermatozoa in the epididymides	composition and concentration of xylene unknown (cotton was soaked in 20 ml of xylene and places in inhalation chamber)
Washington *et al*. (1983)	Sprague Dawley rats(M:(n=?)	dose was given over 2 consecutive d. sacrifice 5 w after exposure	0,5 or 1.5 ml o-xylene/kg bw over 2 d by ip injection	not presented	0.5 and 1.5 ml (room temperature 20-24^{O}C): no effects 0.5 ml (room temperature (24-30^{O}C): reduced spermatogenesis	

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Table 2.1 Development toxicity studies in animals with xylene.

authors	species	experimental period / design	dose and route	general toxicity	development toxicity	remarks
Hudak & Ungvary (1978)	CFY rats (F: n=28 control and n=20 xylene group)	treatment from GD 9-14 sacrifice on GD 21	0 or 1000 mg/m^3 xylene (mix), 24 h/d by inh	no effect on bw	no increase in foetal loss and no effect om foetal weight *1000 mg/m^3* increased fused sternebrae and extra ribs	number of foetuses in xylene group was higher than in control group
Ungváry *et al*. (1980)	CFY rats (F: n=15 control and n=20 xylene groups)	treatment from GD 7-14 sacrifice on GD 21	0, 150, 1500 or 3000 mg/m^3 o-, m- or p-xylene, 24h/d. bij inh	*o-xylene:* increased liver weight in all groups; *m-xylene*: 3000 mg/m^3 decreased bw (on GD 21) and 4 dams died	*1500 mg/m^3 o-xylene:* decreased foetal weight *3000 mg/m^3 o-xylene*: 2 dams with total resoptions; skeletal retardation, decreased foetal weight *3000 mg/m^3 m-xylene*: decreased foetal weight, extra ribs *150 and 1500 mg/m^3 p-xylene*: skeletal retardation *3000 mg/m^3 p-xylene*: 7 dams with total resoprtions, increased foetal loss, decreased foetal weight, extra ribs, skeletal retardation	
Ungaváry *et al*. (1981)	CFY rats (F: n=20)	treatment on GD 9 or GD 9-10 sacrifice GD 11	0 or 3000 mg/m^3 xylene, 24h/d bij inh	not presented	48 h exposure decreased foetal bw.	
Bio dynamics (1983)	Sprague Dawley rats (M: n=10-30; F: n=20-60)	131 days premating, during mating and GD 1-20 1) sacrifice GD 21 2) sacrifice PN d 21 and 49	0.261 1088 or 2175 mg/m^3 xylene (mix), 6h/d by inh	no treatment related effects except for increased bw of females of 261 and 1088 mg/m^3 group during mating	no effect on the number of implantation sites, live foetuses, sex, incidence of foetuses showing delayed development or malformations. No effects during lactation exceot for decreased pup bw in 2175 mg/m^3 group on PN d 49.	

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Table 2.2 Development toxicity studies in animals with xylene

authors	species	experimental period/design	dose and route	general toxicity	development toxicity	remarks
Mirkova *et al*. (1983)	Wistar rat (F: n=46 control and n=160 divided between 3 xylene groups	treatment from GD 1-21 sacrifice part of the animals on GD 21, the others were allowed to litter	0, 10, 50 or 500 mg/m^3 xylene by inh for 6h/d, 5d/w	not presented	*50 and 500 mg/m^3* increased postimplantation loss, decreased foetal weight. Increased incidence of delayed skeletal development. Decreased pup bw and altered activities of metabolic enzymes of pups. *500 mg/m^3* : increased incidence of anomalies of internal organs. Delayed performance in behavioural test.	poorly reported study. Composition of xylene not presented. In 50 and 500 mg/m^3 groups increased incidence of haemorrhages (45% and 53% respectively) although incidence in control group was also high (31%)
Shigeta *et al*. (1983)	ICR mice (F: n=?)	treatment from GD 6-12 sacrifice 2/3 of animals on GD 17, the other were allowed to litter	0, 2175, 4350, 8700 or 17400 mg/m^3 xylene by inh for 6h/d	not presented	no effect on the number of implantation sites resorptions or number of live and dead foetuses. At *8700 and 17400 mg/m^3* foetal bw decreased. Dose related increase in incidence of delayed ossification of sternum and in development of 14th rib. Postnatally, at 17400 mg/m^3 decreased bw and delayed development of hair and teeth	abstract. Xylene undefined.

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Table 2.3 Development toxicity studies in animals with xylene.

authors	species	experimental period/design	dose and route	general toxicity	development toxicity	remarks
Ungvary & Tatrai (1985)	CFY rats (F: n=20 control and n=19-23 xylene groups)	treatment GD 7-15 sacrifice GD 21	0, 250, 1900 or 3400 mg/m^3 xylene (mix). 24 h/d by inh	moderate dose dependent toxic effects (not specified). *3400 mg/m^3*: 1 dam died	*250 and 1900 mg/m^3*: increased skeletal retardation *3400 mg/m^3*: increased no. of resorptions, skeletel retardation and extra ribs and decreased foetal weight	
Ungvary & Tatrai (1985)	CFLP mice (F: n=115 control and n=15-18 xylene groups)	treatment from GD 6-15 sacrifice on GD 18	0 or 500 mg/m^3 o-, m- or p-xylene or 500 or 1000 mg/m^3 xylene (mix), 3x 4h/d by inh	not presented	*500 mg/m^3* (o-, m- and p-xylene): decreased foetal weight and increased skeletal retardation *500 mg/m^3 (mix)* no effects *1000 mg/m^3 (mix)*: decreased foetal weight and increased skeletal retardation.	
Ungvary & Tatrai (1985)	New Zealand Whith rabbits (F: n=60 controls and n=8-10 xylene groups)	treatment from GD 7-20 sacrifice on GD 30	0 or 500 mg/m^3 o-, m- or p-xylene or 500 or 1000 mg/m^3 xylene (mix), 24h/d bij inh	*1000 mg/m^3 p-xylene*: 1 dam died; 3 dams aborted *1000 mg/m^3 xylene* (mix): 3 dams died, 6 dams aborted; increased liver weight	*500 mg/m^3 o-xylene*: no effects *500 mg/m^3 m-xylene* increased no, of respoptions *500 mg/m^3 p-xylene*: no effects *1000 mg/m^3 p-xylene*: 4 dams with total resoprtions; no live foetures at Caesarian section due to death of dams and obortions *500 mg/m^3 xylene* (mix): decreased foetal (female)weight *1000 mg/m^3 xylene* (mix): 1 dam with total resorptions; no live foetuses at Caesarian section due to death of dams and abortions	

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Table 2.4 Development toxicity studies in animals with xylene.

authors	species	experimental period/design	dose and route	general toxicity	development toxicity	remarks
Rosen *et al*. (1986)	Sprague Dawley rats (F: n=25)	treatment from GD 7-16 sacrifice PN d 65	0, 3500 or 7000 mg/m^3 p-xylene, 6h/day by inh	*7000 mg/m^3* reduced bw during exposure period	*7000 mg/m^3* : no effects on weight, viability and motor activity and acoustic startle response of offspring	
Hass & jacobsen (1993)	Wistar rats (F: n=36)	treatment from GD 4-20 1) sacrifice GD 21 (n=24) 2) sacrifice PN d 28 (n=12)	0 or 870 mg/m^3 xylene (mix) 6h/d by inh	no maternal toxicity, no effect on bw	no effects on reproduction and litter data, delayed ossification of os maxillare, higher pup body weight, delayed ear unfolding and eye opening, impaired motorability (Rotarod test)	
Hass (1995)	Wistar rats (F: n=13 control and n=15 xylene group)	treatment from GD 7-20 sacrifice PN d 28	0 or 2175 mg/m^3 xylene (mix) 6h/d by inh	no maternal toxicity, no effect on bw	*2175 mg/m^3* : delay in the ontogeny of the air righting reflex, lower absolute brain weight, impaired performance on motorability, learning and memory test	effects most marked in female offspring
Hass *et al*. (1997)	Wistar rat (F: n=13 control and n=15 xylene group)	treatment from GD 7-20 sacrifice date not presented.	0 or 2175 mg/m^3 xylene (mix) 6h/d by inh	not presented	impaired performance on learning and memory test at 16 and 28 w. PN and not stat sign at 55 w PN.	indication that effect is reversible
Kükner *et al*. (1997)	wistar rat (F: n=5)	treatment from GD 6-21 sacrifice immediately after birth	0 and 11284 mg/m^3 by inh 8h/d	no effect on bw structural changes in the liver at the (ultra)micros-copical level	structural changes in the liver at the (ultra)microscopical level	altered activities of hepatic enzymes of dams

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Table 2.5 Development toxicity studies in animals with xylene.

authors	species	experimental period/design	dose and route	general toxicity	development toxicity	remarks
Nawrot *et al*. (1980)	CD-1 mice (F: n=?)	treatment with unlabeled xylene from GD 6-15 or 12-15. On GD 12 or 15 treatment with ^{14}C-xylene sacrifice 2, 6.5 and 24 h after ^{14}C-xylene treatment	1 ml/kg bw m-xylene and ^{14}C m-xylene by gavage	not presented	in foetal tissue the higest amout of radioactivity was observed 2 h after exposure but at 24 h very little if any radioactivity could be found. 95% of radioactivity was due to metabolites rather than m-xylene itself	abstract
Marks *et al*. (1982)	CD-1 mice (F: n=66 control, n=24-38 xylene groups)	treatment from GD 6-15 sacrifice on GD 18	0.52, 1.03, 2.06, 2.58, 3 10, or 4.13 g xylene (mix)kg bw/d by gavage	*2.06 and 2.58 g/kg* increased maternal liver weight *3.10 g/kg*: 12 dams out of 38 died, increased maternal liver weight *4.13 g/kg*: all dams died	*2.06 and 2.58 g/kg*: increased incidence of cleft palate, open eye, wavy ribs, decreased foetal weight *3.10 g/kg*: increased no. of resorptions, decreased foetal weight	

bw=body weight; h=hours; d=day; w=week; inh=inhalation; mix=mixture of isomers; n=numer of animals; M=males; F=femals; GD=gestation day; PN=post-natal

Annex **E**

Calculation safe levels of xylene in (human) breast milk

Assumptions:

Body weight woman: 60 kg
Body weight infant: 4.5 kg (4-5 kg)
Intake breast milk: 900 ml (800-1000 ml)
An infant is as sensitive for the effects of xylene as an adult.

The Nationale MAC-lijst 2000 (MAC99) proposed a MAC value for xylene of 210 mg/m^3.
A MAC value of 210 mg/m^3 results in a calculated intake of 2100 mg/person/day and 35.0 mg/kg body weight/day.
Maximum permissible intake level per infant is 157.5 mg/infant/day.
Maximum permissible level of xylene in breast milk is 175 mg/l.

In conclusion, the committee considers 175 mg xylene/l breast milk as a maximal permissible level.

Annex **F**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
F	=	female(s)
i.p.	=	intraperitoneal
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no observed adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal