Toluene

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

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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 tolueen. 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

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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. 2001/09OSH, 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.

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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 tolueen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn, en dat voldoende diergegevens laten zien dat tolueen de fertiliteit niet schaadt. Daarom adviseert zij tolueen niet te classificeren.
- Voor ontwikkelingsstoornissen adviseert de commissie tolueen 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 de lactatie adviseert de commissie om tolueen 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 toluene.

The committee's recommendations are

- For effects on fertility the committee recommends not to classify toluene on the basis of a lack of sufficient human data, and sufficient animal data which show that no classification is indicated.
- For developmental toxicity, the committee recommends to classify toluene in category 3 (*substances which cause concern for humans owing to possible developmental effects*) and to label toluene 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 toluene 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 toluene 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 the 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**

Toluene

2.1 Introduction

Name	:	toluene
CAS-no	:	108-88-3
Synonyms	:	toluol, methylbenzene, methylbenzol, phenylmethane
Examples of use	:	Toluene is used in the manufacture of several organic compounds, as a solvent and thinner for paint, glues, varnish and rubber and as a cleaning agent. Toluene is used as a substance of abuse (glue and paint sniffing).
Mol weight	:	92.13
Chem formula	:	$C_6H_5CH_3$
Conversion factor	:	1 ppm = 3.75 mg/m^3 at 760 mm Hg and 20 °C

2.2 Human studies

In the studies described below concerning human exposure to toluene (occupational or by toluene abuse) men are often exposed to a mixture of organic compounds (solvents) containing toluene.

Fertility

Suzuki *et al.* (Suz83) reported a case study on a 28-year-old male who died from excessive thinner sniffing during 10 years. Analysis of his blood revealed a toluene concentration of 43 µg/ml. At autopsy, the testes were imperceptible on naked-eye examination. On microscopic examination, testes were atrophic. The thickened tubular basement membranes were lined with degenerative changes of spermatogonia and Sertoli cells. The seminiferous tubules showed faulty or suppressed spermatogenesis and became smaller and further apart.

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 was slightly, not statistically significantly, higher among wives of man occupationally exposed to toluene (cases n=48, referents=83; crude odds ratio 1.5; CI 0.9-2.5). However, the odds ratio for high or frequent exposure (daily handling of toluene or if biological measurements indicated clear occupational exposure) to toluene was 2.3 (cases n=28, referents n=29; CI 1.1-4.7).

Ng (Ng92a) studied the association between high toluene exposure (mean 88 ppm [330 mg/m^3], range 50-150 ppm [~188 - 562 mg/m^3]) and menstrual disorders in 231 female production workers in a factory manufacturing audio speakers. The women in this group were exclusively exposed to toluene and were employed an average of 6 years in the factory. Control groups were a group of 58 production workers in other departments of the same factory who had little or no exposure to toluene (0-25 ppm [~94 mg/m^3]) and an external group of 187 working class women. No effects of toluene on the rate of menstrual disorders could be established although dysmenorrhoea occurred more often in the groups of women working in the audio speakers factory (Ng92a).

The effect of toluene on hormone levels in 20 toluene-exposed rotogravure printers and 44 unexposed referents was studied by Svensson *et al.* (Sve92a). The mean individual time-weighted toluene level in air was 36 ppm (135 mg/m^3) (range 8-111 ppm [~30 - 416 mg/m^3]). The median levels of toluene in blood and in subcutaneous adipose tissue of the printers were 1.7 ppm (~1.7 mg/l) (range 1.0-6.6 ppm [1.0-6.6 mg/l]) and 5.7 ppm (5.7 mg/kg) (range 2.5-21 ppm [2.5-21 mg/kg]), respectively. Median plasma

levels of follicle stimulating hormone (3.2 IU/l in toluene-exposed group vs. 4.9 IU/l in control group), luteinizing hormone (6.4 IU/l in toluene-exposed group vs 7.2 IU/l in control group) and serum levels of free testosterone (76.8 pmol/l in toluene-exposed group vs. 86.8 pmol/l in control group) were lower in the toluene exposed group. Furthermore, there was a negative association between blood toluene levels and plasma levels of prolactin. The effects on follicle stimulating hormone and luteinizing hormone were reversible since the plasma levels of these hormones were increased after a 4 week vacation whereas the levels of thyroid stimulating hormone, free triiodothyronine and free thyroxine were decreased during the same period.

In another study of this group (Sve92b) the effect of toluene (air toluene concentration ranged from 6-142 ppm [~23-533 mg/m^3] and blood toluene concentration ranged from 0.19-7.99 µmol/l [~0.02-0.74 mg/l]) on plasma levels of testosterone, prolactin (PRL) and luteinising (LH) and follicle stimulating (FSH) hormones was investigated in 47 rotogravure printers and in a reference group. Increasing concentrations of toluene were significantly associated with decreasing concentrations of LH and testosterone. There was no correlation between cumulative exposure and plasma hormone concentrations. The authors concluded that the results of these studies (Sve92a, Sve92b) indicate an effect of toluene exposure on the hypothalamic-pituitary axis.

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 a 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 toluene were 0.99; 95% CI 0.64-1.53 (low exposure, n=33) and 0.71; 95% CI 040-1.26 (high exposure, n=17). However, the incidence density ratios for women working in the shoe industry, in which the most common solvents used are toluene, hexane and acetone, were 0.28 ; 95% CI 0.11-0.71 (low or high exposure, n=6) and 0.19; 95% CI 0.05-0.79 (high exposure, n=3).

In a prospective study of Lemasters *et al.* (1999) sperm production, structure, and function were evaluated in a total of 50 exposed workers (divided into four groups: sheet metal workers (n=6), painters (n=6), jet fuel workers (n=15) and flight line crew (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 (Lem99). 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 = 22.5 mg/m^3). For most sperm measures, mean values remained in the normal range throughout the 30 weeks exposure period. Men in the paint shop group, with a higher exposure to solvents, had a significant decline in sperm motility of 19.5% at 30 weeks, however there was no significant association between internal dose and spermatogenic changes. The authors stated that the lack of significance but also the positive findings could be related to inherent variability in the measures or to chance due to the small subgroups (Lem99).

Women in the follicular (n=5) and luteal phases (n=6) of menstrual cycle and men (n=5) were randomized to inhaled filtered air with 50 ppm (187.5 mg/m^3) toluene or to filtered air alone (control n=5 per group) for 3 hours (Lud99). Blood was sampled by intravenous catheter at 20 minutes intervals for 3 hours before, 3 hours during and 3 hours after exposure. Mean concentrations of LH and FSH were measured. No abnormal episodic LH or FSH secretion profiles were observe, however, subtle effects on LH secretion in men and in women in luteal phase were found. The clinical relevance of these effects is unclear.

In a cross sectional study of Plenge-Bönig and Karmaus (Ple99) 150 male and 90 female printing industry workers were interviewed retrospectively on reproductive experience. After considering possible biases, low daily exposure to toluene in women seems to be associated with reduced fecundity (fecundity ratio 0.47; 95% CI 0.29-0.77). Toluene exposure of male workers did not result in an effect on fecundity (fecundity ratio 1.05; 95% CI 0.93-1.19).

Development

Holmberg (Hol79) and Holmberg *et al.* (Hol80) identified 132 cases of children with congenital central nervous system defects in two-year material 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 in both and 3 referent mothers reported exposure to (various) organic solvents 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 cases 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).

Toutant and Lippman (1979) described the birth of a child to a 20 year old primigravida whose major addiction was to solvents (primarily toluene) for 14 years. On admission, she exhibited signs compatible with severe solvent and/or alcohol abuse. The child was born at term, was small and exhibited abnormal features that included microcephaly, a flat nasal bridge, hypoplastic mandible, short palpebral fissures, mildly low-set ears, pronounced sacral dimple, sloping forehead and uncoordinated arm movements (Tou79).

In two studies of Hersch *et al.* (Her85, Her89) 5 cases are described of chronic maternal toluene inhalation abuse before and throughout pregnancy. There was no evidence of excessive use of other (teratogenic) substances. The most frequently described clinical features in these children are microcephaly, central nervous system dysfunction, attentional deficits or hyperactivity or both, developmental delay with greater language impairment and growth retardation. Common phenotypic abnormalities in these children were small midface, narrow bifrontal diameter, short palpebral fissures with deep set eyes, low set ears, flat nasal bridge with a small nose, micrognathia and blunt fingertips. The authors considered these effects as features of toluene embryopathy.

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 toluene exposure was 1.6; 95% CI 0.6-4.5). The incidence of spontaneous abortion depended on the frequency of exposure; odds ratio was 1.2 (95% CI 0.2-6.9) if toluene exposure occurred less than once a week and odds ratio was 1.9 (95% CI 0.6-6.4) if toluene exposure occurred more than once a week.

In a case-referent study of McDonald *et al.* (Don87) 301 women who in their most recent pregnancy had given birth to an infant with an important congenital defect were individually matched with 301 women whose children were normal. Occupational exposure to chemicals was investigated and only exposure to aromatic solvents and especially to toluene showed a clear effect, most evident in renal-urinary tract and gastrointestinal tract defects.

Goodwin described 5 cases of pregnant women with severe renal tubular acidosis due to sniffing of toluene containing paint (0.5-2 cans of paint for 6 months -3 years)

(Goo88). Two of the infants showed hyperchloremic acidosis (resolving within 72 hours) and anomalies. Three of 5 infants were growth-retarded at birth. A comparable case of a mother sniffing paint containing toluene during pregnancy and giving birth to a premature infant with renal tubular acidosis was described by Erramouspe *et al.* (Err96).

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 for toluene exposure was 1.6; 95% CI 0.7-3.8 (15 cases and 20 controls). The odds ratio of spontaneous abortion due to toluene exposure decreased with the level of exposure; the odds ratios for low and high exposure were 1.8; 95% CI 0.7-4.7 (10 cases and 11 controls) and 1.4; 95% CI 0.4-4.9 (5 cases and 8 controls), respectively. The odds ratio of spontaneous abortion in the shoe industry, in which the most common used solvents were toluene, hexane and acetone, was 9.3; 95% CI 1.0-84.7 (5 cases and 2 controls).

Wilkins-Haug *et al.* (Wil91) reported 10 cases of women with chronic glue- and paint sniffing abuse (mean duration of toluene abuse was 7.9 years). Thirty pregnancies were reported by the ten women, from which 21 toluene-exposed infants were delivered. Preterm delivery, perinatal death and growth retardation were significantly increased. Growth retardation and developmental delay were common findings in these children.

Ng *et al.* (Ng92b) determined the rates of spontaneous abortions using a reproductive questionnaire administered by personnel interview to 55 married women with 105 pregnancies. They were employed in an audio speaker factory and were exposed to high concentrations of toluene (mean 88 ppm [330 mg/m^3], range 59-150 ppm [~221-563 mg/m^3]). The rates of spontaneous abortion were compared with those among 31 women (68 pregnancies) who worked in other departments in the same factory and had little or no exposure to toluene (0-25 ppm [~94 mg/m^3]) as well as with a community control group of women who underwent routine antenatal and postnatal care at public maternal health clinics (190 women with 444 pregnancies). The incidences of spontaneous abortions were statistically significantly higher in the group with high exposure to toluene (12.4 per 100 pregnancies) compared with those in the internal control group (2.9 per 100 pregnancies) and in the external control group (4.5 per 100 pregnancies).

Arnold *et al.* (Arn94) reviewed the case reports of 35 deliveries from toluene-exposed pregnancies in 15 women. There were 3 perinatal deaths. In the

remaining infants a high incidence of prematurity (42%), low birth weight (52%) and microcephaly (32%) was observed. Follow-up pediatric evaluation revealed growth retardation, microcephaly and developmental delays. Six children which were examined demonstrated microcephaly, narrow bifrontal diameter, short palpebral fissures, hypoplastic midface, wide nasal bridge, abnormal palmar creases and blunt fingertips.

Pearson *et al.* (Pea94) examined 18 infants with a history of in utero toluene exposure (due to toluene abuse) at birth and 9 of them were re-examined 3 to 36 months after their initial evaluations at birth. In this study 39% of the infants were born prematurely, 33% were small for gestational age, 33% exhibited continued postnatal growth deficiency. The incidence of prenatal microcephaly was 28%, of postnatal microcephaly 89% and of developmental delay 83%. Furthermore, a high incidence of craniofacial features and other minor anomalies was observed.

Spontaneous abortions among women working in laboratories, and congenital malformations and birth weight of the children were examined in a retrospective case-referent study of Taskinen *et al.* (Tas94). Frequent exposure to toluene (at least 3 days a week) was statistically significantly associated with spontaneous abortion (cases n=10, referents n=6, odds ratio 4.7; 95% CI 1.4-15.9). No association with birth weights or incidence of congenital malformations was observed.

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 toluene (50 ppm = 188 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 toluene ingested by a nursing infant over a 24-hour period was 0.46 mg in 0.92 l (0.5 mg/l).

Based on the proposed ADI of 430 µg/kg body weight/day (RIVM: Jan94), the maximal permissible level of about 2.2 mg/l breast milk, can be calculated for toluene (see Annex E).

2.3 Animal studies

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

Fertility studies

Nylen *et al.* (1989) found no alterations in testes, accessory glands or circulating male hormone levels in male Sprague Dawley rats after inhalation exposure to 1000 ppm (3750 mg/m^3) toluene for 28 days, 21 hours a day for 7 days a week. General toxicity was not described (Nyl89).

In a study of Yamada (Yam93) the effect of toluene on the reproductive and accessory reproductive organs of male Wistar rats was studied. Exposure concentration is not described; cotton was soaked in 20 ml of toluene and placed in the inhalation chamber. The animals were kept in the inhalation chamber until anaesthesia was achieved after about 4-6 min twice a day for 7 days. There was no effect of toluene on body weights. Furthermore, there was no effect on the weight of the testes, epididymis, vas deferens, seminal vesicles and prostate and on plasma testosterone levels and acid phophatase activity in the prostate. Also the number of spermatozoa in the epididymis was not affected by toluene.

Ono *et al.* (Ono96) exposed male and female Sprague-Dawley rats to toluene (0, 600 and 2000 ppm; 0, 2250, 7500 mg/m^3) by inhalation for 6h/d. Females were exposed from 14 days premating until gestation day 7 (GD 7), males were exposed for a total of 90 days, including the mating period. On GD 20, all pregnant females were sacrificed for foetal examination. In the females of the 2000 ppm group, 20 days after starting dosing salivation and lacrimation was observed. Body weights and food consumption were slightly decreased. No effects were observed on mating and fertility indexes. Foetal mortality and the number of dams with dead fetuses were increased in the 2000 ppm group. In the males of the 2000 ppm group, an increase in kidney weight and a decrease in thymus weight were observed. Furthermore, the weight of the epididymides was decreased and a decrease in spermatic count was observed. No effects were observed on testis and epididymides morphology and on the number of spermatogenic cells at stages V, VIII and XII in seminiferous tubules.

Sprague Dawley rats were exposed to toluene (0, 4000, 6000 ppm = 0, 15000, 22500 mg/m^3) 2 h/day, 7 days/week for 5 weeks to determine whether toluene abuse affects the reproductive functions or general health (Ono99). All exposed rats exhibited excessive salivation and lacrimation and body weights and food consumption were dose-relatedly decreased. Furthermore, rats exposed to 6000 ppm had decreased spleen and thymus weights as well as suppressed lymphocyte count. In the 6000 ppm group, the number of epididymal sperm cells, sperm motility, sperm quality and in vitro sperm penetration rate were reduced. Since there were no effects observed on the testis weight, spermatogenesis process within the testis and on serum hormone levels, the authors concluded that high concentrations of toluene may directly target sperm in the epididymis and disrupt sperm maturation.

Developmental studies

Inhalation

Hudák and Ungváry (Hud78) exposed three groups of CFY-rats to toluene by inhalation. The first group (n=19) received 1500 mg/m^3 during 24 h/day from GD 9-14. Two of 19 treated animals died. No effects were observed on maternal body weights. Toluene treatment did not appear to be foetal-toxic. Foetal development was normal except for an increase in irregular and fused sternebrae and extra ribs and two foetuses with missing tails. The second group (n=9) received the same exposure to toluene but only on the first 8 days of gestation. In this case, there was a high incidence of maternal mortality, 5 of the 14 dams died and a decrease in foetal weight was noted. A significant retardation in skeletal development was observed with shortening of the 13th ribs. The third group (n=10) was exposed to a lower dose of 1000 mg/m^3 during 8 h/day from GD 1-21. There was no maternal or foetal weight loss. There were no malformations in these foetuses but a significant retardation in skeletal development was observed. In the same study CFLP-mice (n=11) were exposed to 1500 mg/m^3 24 h/day from GD 6-13. This concentration was lethal to all dams. In a group of mice exposed to 500 mg/m^3 24 h/day from GD 6-13 no maternal lethality and no malformations other than in the control group were observed. Foetal body weights were decreased in this group.

CFY rats were exposed to 0 (n=22) or 1000 mg/m^3 (n=20) toluene by inhalation for 24 h/day from GD 7-14. On GD 21 rats were sacrificed for foetal examination. No maternal toxic effects of toluene exposure were observed. Toluene exposure increased the incidence of skeletal retarded foetuses. Except for a small increase in foetuses with an extra rib no additional anomalies or malformations were induced by toluene (Tát80).

Pregnant ICR-mice were exposed by inhalation to toluene (0, 100 and 1000 ppm; 0, 375, 3750 mg/m^3) for 6h/day from GD 1-17 (Shi82). On GD 18, 2/3 of the mice of each group were sacrificed for foetal examination and the other mice of each group were allowed to litter. Except for an increase in the incidence of extra 14th ribs in the 1000 ppm group there were no effects of toluene on the incidence of mating and fertility indexes, external malformations and development of the offspring. Maternal toxicity was not presented.

Ungváry and Tátrai (Ung85) exposed CFLP mice and NZ rabbits by inhalation to 0, 133 and 266 ppm (0, 500, 1000 mg/m^3) toluene for 24 h/day from GD 6-15 or 7-20, respectively. Mice or rabbits were sacrificed for foetal examination on GD 18 or 30, respectively. Body weights of mouse foetuses exposed to 266 ppm were decreased and the incidence of skeletal retarded foetuses was increased. Maternal toxicity was not presented. In the group in which mice were exposed to 1500 mg/m^3 toluene, all dams

died. In the highest dose group of the rabbits, maternal body weights were decreased and the incidence of spontaneous abortions was increased.

Courtney *et al.* (Cou86) administered toluene by inhalation (0, 200 and 400 ppm; 0, 750, 1500 mg/m^3) to CD-1 mice from GD 7-16 for 7h/day. Mice were sacrificed on GD 17. Maternal body weights were not affected by toluene treatment whereas relative liver weights were decreased. There were no differences between the control and toluene-exposed groups in the number of implantation sites, number of live foetuses, foetal mortality and foetal body weight. Examination of the foetuses revealed that toluene increased the number of foetuses showing dilated renal pelves (200 ppm group but not in the 400 ppm group). The number of foetuses with 14 ribs was slightly but not statistically significantly increased in the 200 ppm group and the number of foetuses with 13 ribs was statistically significantly increased in the 400 ppm group. In a postnatal part of this study, CD-1 mice were treated with toluene by inhalation (0 and 400 ppm; 0 and 1500 mg/m^3) from GD 7-16 for 6 h/day (Cou86). The mortality rate at birth was not statistically significantly increased by toluene. Body weights of pups on postnatal day 1 (PN day 1) was statistically significantly increased in the toluene-exposed group, on PN day 21 this increase was no longer statistically significant.

Shigeta *et al.* (Shi86) treated nonselect Wistar rats and HA rats (high- and stable-avoidance and low-variability rats) with 100 ppm (375 mg/m^3) toluene for 7 h/d from GD 13 to PN day 48. Maternal toxicity was not presented. There were no effects of toluene on developmental parameters in both Wistar and HA rats. Foetal body weight gain was larger in the toluene treated rats than in the control rats. Except for the male HA rats, there was no effect of toluene on learning capability of the Sidman avoidance test.

In a study of da-Silva *et al.* (Sil90b), Wistar rats and hamsters were exposed to toluene by inhalation (800 mg/m^3) for 6 h/d from GD 14-20 and GD 6-11, respectively. Subsequently, growth, neuromotor development and performance of the offspring in behavioural tasks were assessed. In both species, no maternal toxicity was observed. In rats the number of pups born alive was decreased and the number of litters with pups with a low birth weight was increased. Male rat pups displayed shorter latencies in a spontaneous alternation test and on one of the three test days pups of hamsters performed worse in a rotating test compared with control pups.

Klimisch *et al.* (Kli92) determined the prenatal toxicity of toluene in Himalayan rabbits in two separate studies. In the first study, rabbits were treated with 30, 100 and 300 ppm (113, 375, 1125 mg/m^3) toluene by inhalation for 6 h/day from GD 6-18 and in the second study with 100 and 500 ppm (375, 1875 mg/m^3) for the same time period. No signs of maternal toxicity were observed and all reproduction data were found to be within the variation range reported for this rabbit strain. The incidences of foetal

external, -soft tissue and -skeletal findings which were observed in toluene exposed foetuses were similar to the corresponding and/or historical controls.

Ono *et al.* (Ono95) treated Sprague-Dawley female rats with 0, 600 and 2000 ppm (0, 2250, 7500 mg/m^3) by inhalation for 6 h/day from GD 7-17. Part of the rats were sacrificed on GD 20 for foetal examination, the remaining females were allowed to litter for pre- and postweaning behavioural tests. In the 2000 ppm group, body weights and food intake of the dams were statistically significantly decreased during the exposure period but not thereafter. In the offspring, body weights were decreased and a high foetal mortality and embryonic growth retardation was observed whereas there was no effect after exposure to 2000 ppm on external, internal and skeletal anomalies in the foetuses. Furthermore, there was no effect on pre- and postweaning behavioural. In the 600 ppm group there were no toxic or teratogenic effects observed.

Jones and Balster (Jon97) studied the effects of toluene on physical and behavioural development in CD-1 mice prenatally exposed to 0, 200, 400 or 2000 ppm toluene (0, 750, 1500, 7500 mg/m^3) by inhalation for 60 min, 3 times a day during GD 12-17 (exposure regimen simulates human exposure that might occur with toluene abuse). No maternal toxicity was observed. Gestation length, number of litters and litter size were not affected. Pups were evaluated on PN days 1-20. In the 2000 ppm group the individual pup weights were decreased and the pups performed worse in various behavioural tests. In the 200 and 400 ppm groups no effects of toluene were observed.

Thiel and Chahoud (Thi97) exposed pregnant Wistar rats to 0, 300, 600, 1000 and 1200 ppm (0, 1125, 2500, 3750, 4500 mg/m^3) toluene by inhalation for 6 h/day during GD 9-21 where after they were allowed to litter. At 1200 ppm, body weights of dams and offspring were reduced and a higher mortality of the pups was observed. Physical development was retarded in this group. No effects on development and learning ability were observed. Furthermore, there were no effects on mating, fertility and pregnancy indices in the F1-generation.

Hass *et al* (Has99) exposed female rats (Mol:WIST) by inhalation to 0 and 1200 ppm (0 and 4500 mg/m^3) toluene for 6 hours per day from day 7 of pregnancy until day 18 postnatally. Developmental and neurobehavioral effects in the offspring were investigated. The exposure did not cause decreased viability in the offspring. Lower fetal birth weight, delayed ontogeny of the reflexes and increased motor acitivity was registered. No maternal toxicity was found (but not well studied) except for a decreased maternal weight.

In a recent study of Hougaard *et al.* (Hou99), the effects of prenatal exposure to toluene on postnatal development and behaviour in rats were studied. Pregnant rats were exposed to 0 and 1800 ppm (0, 6750 mg/m^3) toluene by inhalation for 6 hours daily during GD 7-20. Except for a slight, not significant, decrease in body weights of the dams exposed to toluene no signs of maternal toxicity was observed. No significant

effects on reproduction were found and no pups with external malformations were observed. Body weights of the pups were decreased by toluene exposure up to PN day 10. Neurobehavioral evaluation of the pups revealed no effects on motor function (rotarod), activity level (open field), acoustic startle and prepulse inhibition. Small effects were observed in hearing function of male pups. Cognitive functions were impaired, most marked in female pups, as was measured by Morris water maze performance.

Gavage

In a series of studies of Gospe *et al.* (Gos94, Gos96, Gos98) the effect of toluene on the development of Sprague-Dawley rats was studied.

In a first study, Gospe *et al.* (Gos94) treated Sprague-Dawley rats with toluene (520 mg/kg) by gavage from GD 6-19. Rats were sacrificed on GD 19 for foetal examination. Maternal weight gain in the toluene-exposed group was decreased. There was no effect on the number of implantations, stillbirths, congenital malformations and neuropathological changes. In the toluene-exposed group, foetal-weights, placenta weights and several foetal absolute organ weights were decreased. There was no effect of toluene on foetal relative organ weights, suggesting that toluene induced a generalized growth retardation.

In a second study of this group (Gos96) Sprague-Dawley rats were treated with toluene (650 mg/kg) by gavage from GD 6-19 where after the rats were sacrificed on GD 19 for foetal examination. Maternal body weights and food consumption were decreased. In the toluene exposed group foetal body weights and several foetal organ weights (including brain) were decreased. Furthermore, a delay in skeletal ossification was observed. Morphometric analysis of brain sections demonstrated that toluene exposure resulted in smaller brains going together with an increased size of the ventricular system and a reduction in the size of the caudate nucleus. As was suggested in the previous study of this group, these results indicate that toluene exposure results in generalized foetal growth retardation.

In a third study of Gospe *et al.* (Gos98), Sprague-Dawley rats were exposed to toluene (650 mg/kg) by gavage from GD 6-19. Litters were evaluated on GD 19 and on PN day 10 and 21. Maternal toxicity was not presented. Prenatal toluene exposure induced foetal growth retardation with foetuses showing smaller brains and caudate-putamen volumes, fewer forebrain cell nuclei and a reduction in both hindbrain cell size and myelination per cell. However, since postnatal growth progressed until PN day 21 these differences had resolved except for a toluene induced reduction in myelination per cell of the forebrain which appeared to be permanent.

Drinking water

The effects of toluene on several behavioural parameters were examined in mice which were exposed to toluene (16, 80 and 400 mg/l in drinking water) during the mating, gestation and lactation period. The offspring was exposed continuously from weaning at PN21 through behavioural testing (Kos81). At 35 days of age decreased habituation of open-field activity was seen in the 400 mg/l group whereas rotorod performance, measured at 45-55 days of age, was depressed in all exposed groups. No effect of toluene was observed on maternal mortality, body weights and water consumption, offspring mortality, development of eye, ear openings or surface-righting response.

Subcutaneous

Pregnant Wistar rats were subcutaneously treated with toluene (1.2 g/kg) during GD 8-15 or GD 14-20 (Sil90a). In rats treated from GD 8-15, body weights were decreased during the period of toluene administration. There was no effect of toluene on the incidence of malformations nor on skeletal growth. Toluene exposure during GD 14-20 resulted in decreased maternal body weights during the period of toluene administration and decreased foetal body weights at birth which persisted in the male offspring into adulthood. No effects of toluene were observed on behavioural tests performed when pups were 30 and 95 days old.

Lactation

In a study of da-Silva *et al.* (Sil91) the effect of exposure of rat offspring to toluene through maternal milk on growth and development was investigated. Lactating Wistar rats were subcutaneously treated with toluene (1.2 g/kg body weight) daily from lactation day 2-21. No effects of treatment on offspring development or on any of the behavioural tests were observed. Another group of rats was treated subcutaneously with a single dose of 1.2 g toluene/kg body weight on lactation day 10. Toluene levels in milk measured 4 hours after the single injection were 5 times higher than in blood; 10.3 ± 6.2 mg/100 ml milk vs. 2.1 ± 0.8 mg/100 ml blood, respectively.

2.4 Conclusion

In a case report, Suzuki *et al.* reported a very small testis in a man who died from excessive thinner sniffing (Suz83). The incidence of spontaneous abortions among wives of men occupationally exposed to toluene was slightly, not statistically significantly, increased (Tas89). However, in a subgroup of men highly or frequently

exposed to toluene the effect was more pronounced. Among women exposed to toluene, no endocrine effects were observed (the clinical relevance of the subtle effects on LH secretion in men and women in luteal phase is unclear) (Lud99). No effect of toluene exposure was observed on the incidence of menstrual disorders (Ng92a). Plenge-Bönig and Karmaus (Ple99) reported a reduced fecundity in women after low daily toluene exposure whereas in the study of Sallmén *et al.* (Sal95) no effect of toluene exposure on fecundity was observed. In man, occupational toluene exposure affected plasma levels of various hormones (Sve92a, Sve92b). In the study of Lemasters *et al.* (Lem99), only the effects of exposure to a mixture of solvents on sperm parameters were studied.

Although effects were observed on fertility in men, it is not clear whether the described effects are due to toluene exposure alone or to other compounds present in the working place or during abuse. For this reason, to the committee's opinion, a lack of appropriate human data precludes the assessment for fertility.

In Ono *et al.* (Ono96), no effects on mating and fertility of Sprague Dawley rats were observed at dose levels which induce general toxicity. In Ono *et al.* (Ono99) effects on fertility were only observed at dose levels at which general toxicity was observed. In the study of Yamada *et al.* (Yam93), no effects of toluene exposure (the concentration of toluene was not measured but the animals were kept in an inhalation chamber until anaesthesia was achieved after about 4-6 min twice a day for 7 days) on testis and accessory reproductive organs were observed. In the study of Nylen *et al.* (Nyl89), no effects on testes, accessory glands and hormone levels were observed but it is not clear from the data presented if toluene, at the dose level used, induced general toxic effects.

In conclusion, on the basis of the study of Ono *et al.* (Ono96), the committee is of the opinion that sufficient animal data show that no classification is indicated for effects on fertility.

Developmental effects in men after toluene exposure due to glue and paint sniffing abuse during pregnancy were reported by Toutant and Lippman (Tou79), Hersch *et al.* (Her85 and Her89), Goodwin (Goo88), Erramouspe *et al.* (Err96), Pearson *et al.* (Pea94) and Arnold *et al.* (Arn94).

Exposure at the working place was studied by Holmberg (Hol79 and Hol80), McDonald *et al.* (Don87), Lindbohm *et al.* (Lin90), Ng *et al.* (Ng92b) and Taskinen *et al* (Tas86, Tas89 and Tas94). The following effects were reported: children with central nervous system defects (Hol79, Hol80), renal-urinary tract and gastrointestinal defects (Don87), and an increased incidence of abortions (Lin90, Ng92b, Tas89, Tas94).

However, in all studies in man, it is not clear if the described effects are due to toluene exposure alone or to other compounds in the working place or during abuse.

In studies in experimental animals (rats, mice, rabbits, hamsters) toluene caused skeletal retardation, extra ribs, decreased foetal/pup weight effects and/or pup mortality (Hud78, Tát80, Shi82, Ung85, Cou86, Sil90a, Sil90b, Gos94, Ono95, Gos96, Jon97, Thi97, Gos98 and Has99) at or near dose levels which caused also maternal toxicity.

Behavioural effects of toluene exposure during gestation were detected by Shigeta *et al.* (Shi86), da-Silva *et al.* (Sil90), Jones and Balster (Jon97), Hougaard *et al.* (Hou99) and Hass (Has99) at or near maternally toxic dose levels or the maternal toxicity was not well studied.

In conclusion, the committee proposes to classify toluene in category 3 (substances which cause concern for humans owing the possible developmental toxic effects) and to label the compound with R63 (possible risk of harm to the unborn child).

From the study of Fisher *et al.* (a pharmacokinetic lactation model), an amount of 0.5 mg toluene/l breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) toluene 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 toluene in human breast milk and about the possible effects during lactation.

In only one study with animals (Sil91), a relatively high concentration of toluene (10.3+ 6.2 mg/100 ml) in milk of rats was detected 4 hours after a single high dose of 1.2 g/kg body weight (subcutaneously treated on lactation day 10). However, the committee considers this route of exposure less relevant for labelling purposes. In conclusion, the committee recommends not to label toluene for effects during lactation due to a lack of appropriate data.

Proposed classification for fertility

A lack of appropriate human data precludes the assessment of toluene for fertility, and sufficient animal data show that no classification is indicated.

Proposed classification for developmental toxicity

Category 3, R63

Proposed labelling for effects during lactation

Lack of appropriate data precludes the assessment of toluene for effects during lactation.

Additional consideration

The committee would like to emphasize that several human studies considered here in view of toluene exposure give reason for concern with respect to effects on fertility and development. Several authors have described these effects as ‘toluene embryopathy’. However, it is not clear in these studies whether exposure involved pure toluene or a mixture of solvents containing toluene. Therefore, the EU Classification and Labelling guideline does not warrant a classification of toluene 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 toluene.

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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
- A Aalto
  Ministry of Social Affairs and Health, Finland

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 toluene.

authors	species	experimental period/design	dose and route	general toxicity	findings	remarks
Nylen *et al.* (1989)	Male Sprague Dawley rats (n=18)	treatment for 28 d. sacrifice: 2w, 10m, 14m after end exposure	3750 mg/m^3 21 h/d by inhalation	not presented	no effects on testis, accessory sex glands or hormone levels.	
Yamada (1993)	Male Wistar rats (Control group n=20, toluene group n=5)	2 x4-6 min/d for 7 d sacrifice: first day after end of treatment	cotton soaked in 20 ml of toluene was placed in inhalation cylinder. Treatment by inhalation	no effects on bw.	No effects on the weights of testes, epididymis, vas deferens, seminal vesicles and prostate. No effect on plasma testosterone levels and on prostatic phosphatase activity. No effect on the number of spermatozoa in the epididymes.	
Ono *et al.* (1996)	Sprague-Dawley rats (n=15/sex/group)	6h/d Females:14d PM-GD 7 Males: 90 d starting 60 d PM Sacrifice females: GD 20	0, 2250 and 7500 mg/m^3 6 h/d by inhalation	*7500 mg/m^3* Females: salivation and lacrimation; decreased bw and fc. Males: weight of kidney increased and weight of thymus decreased	*7500 mg/m^3 group*: *Females:* no effect on mating and fertility, fetal mortality increased *Males*: weight of epididymides decreased, sperm counts decreased, no effect on testis and epididymides morphology, no effect on the number of spermatogenic cells at stages V, VII, XII in seminiferous tubules	
Ono *et al.* (1999)	Sprague-Dawley rats (n=5)	2h/d, 7d/w for 5 w sacrifice at end of exposure	0, 15000 and 22500 mg/m^3 by inhalation	*15000 and 22500* mg/m^3 salivation and lacrimation; decreased bw and fc. *22500 mg/m^3* decreased spleen and thymus weight	*22500 mg/m^3* : number of epididymal sperm cells, sperm motility, sperm quality and in vitro sperm penetration rate reduced. No effects on testis weight, spermatogenesis process in testis and om serum hormone levels	

bw= body weight; n= number of animale per group; h= hour; d= day; w= week; m= mouth; GD= day of gestation; PM= pre-mating ; fc= food consumption

Table 2.1 Developmental toxicity studies in animals with toluene.

authors	species	experimental period/design	dose and route	general toxicity	developmental toxicity	remarks
Hudák and Ungváry (1978)	CFY-rats 1) control n=28, toluene n=21, 2) control n=28, toluene n=14, 3) control and toluene n=10	1) GD 9-14 2) GD 1-8 3) GD 1-21 sacrifica: GD 21	1) 1500mg/m^3 24h/d 2) 1500mg/m^3 24h/d 3) 1000 mg/m^3 8h/d by inhalation	1) 2 animals died, no effect on bw 2) 5 animals died 3) no maternal loss	1) no effect on fetal body weight, no effect on malformations, increase in irregular and fased sternebrae and extra ribs. 2) decreased fetal weight, no effects on malformations, retarded skeletal development. 3) no effect on malformations, retarded skeletal development.	
Hudák and Ungváry (1978)	CFLP mice control n=14, toluene n=11	GD 6-13 sacrifice GD 18	500 mg/m^3 24h/d by inhalation	no maternal loss	fetal body weight was decreased. No effect on the number of fetuses, no effect on malformations.	1500 mg/m^3 24h/d was lethal to all exposed dams
Tàtrai and Ungváry (1980)	CFY rats control n= 22 toluene n=20	GD 7-14 sacrifice GD 21	0 and 1000 mg/m^3 24h/d by inhalation	no toxic effects observed	no effect on malformations, increased number of fetuses with extra rib, retarded skeletal development	
Kostas and Hotchin (1981)	Nya:NYLAR mice n=12	treatment during mating, pregnancy, lactation and offspring from weaning (PN21) through behavioural testing. Sacrificed ?	0, 16, 80 and 400 mg/I in drinking water	no toxic effects observed	no effect on development of eye or ear openings or surface-righting respons. Rotorod performance was depressed in all exposed groups and open-field activity in 400 mg/I group.	in acutely exposed animals (ip) no effect was observed on open-field activity.

h=hour d= day n= number of animale per group; bw= body weight; PN= postnatal day; GD= gastation day

Table 2.2 Development toxicity studies in animals with toluene.

authors	species	experimental period/design	dose and route	general toxicity	developmental toxicity	remarks
Shigeta *et al*. (1982)	ICR-mice 1) n=15 2) n= 18 3) n=14	GD 1-17 sacrifice GD 18 (2/3 of mice, other were allowed to deliver)	1) 0 mg/m^3 6h/d 2) 375 mg/m^3 6h/d 3) 3750 mg/m^3 6h/d by inhalation	maternal toxicity was not presented	not statistically increased number of resorptions, no effect on implantations, live or dead fetuses, body weights, malformations and development of pups. In 3750 mg/m^3 increased number of pups with extra 14th ribs	
Ungváry and Tátrai (1985)	CFLP mice 1) n=115 2) n= 15 3) n= 15	GD 6-15 sacrifice GD 18	1) 0 mg/m^3 24h/d 2) 500 mg/m^3 24h/d 3) 1000 mg/m^3 24h/d by inhalation	maternal toxicity was not presented	*1000 mg/m^3* : decreased fetal body weights and retarded skeletal development. No effect on malformations	1500 mg/m^3 24h/d all dams died
Ungváry and Tátrai (1985)	NZ rabbits 1) n= 60 2) n= 10 2) n= 8	GD 7-20 sacrifice GD 30	1) 0 mg/m^3 24h/d 2) 500 mg/m^3 24h/d 3) 1000 mg/m^3 24h/d by inhalation	1000 mg/m^3 : bw and liver weight decreased	*1000 mg/m^3* : increased number of spontaneous abortions.	
Courtney *et al*. (1986)	CD-1 mice 1) n= 15 2) n= 16 3) n= 16	GD 7-16 sacrifice GD 17	1) 0 mg/m^3 7h/d 2) 750 mg/m^3 7h/d 3) 1500 mg/m^3 7h/d by inhalation	no effect on bw, relative liver weight was decreased	no effects on the number of implantations, live fetuses and fetal mortality and fetal body weights. *750 mg/m^3* : increased number of fetuses with dilated renal pelvis: Not statistically increased number of fetuses with 14 ribs. *1500 mg/m^3* : statistically significant increased number of fetuses with 13 ribs	

h=hour; d= day; n= number of animals; bw= body weight; GD= gestation day

Table 2.3 Development toxicity studies in animals with toluene.

authors	species	experimental period/design	dose and route	general toxicity	developmental toxicity	remarks
Courtney *et al*. (1986)	CD 1 mice 1) n=9 2) n=8	GD 7-16 sacrifice PN 21	1) 0 mg/m^3 6h/d 2) 1500 mg/mg^3 6h/d by inhalation	maternal toxiciity not presented	not statistically significant increased mortality rat at birth. Fetal body weights on PN 1 were statistically significant increased, no effect on PN 21.	
Shigeta *et al*. (1986)	Wistar rats (n=31-38) HA rats (n=19-23)	GD 13 - PN day 48 sacrificed?	375 mg/m^3 for 7h/d by inhalation	mtoxicity was not presented	no effects on developmental parameters. Foetal body weight gain decreased.	
da-Silva *et al*. (1990a)	Wistar rats 1) n=8 2) n=7 3) n=7 4) n=8	1), 2): GD 8-15 sacrifice: GD 20 3), 4): GD 14-20 sacrifice: PN 1 and PN 95	1), 3): 0 g/kg bw 2), 4) 1.2 g/kg bw by s.c. injection	2) and 4) decreased bw	*2)* no malformations and no effect on development of skeleton. *4)* decreased fetal body weights at birth which persisted in the male offspring into adulthood. No effect on skeletal development. No effects on behavioural test performed on PN 30 and 95	malnutrition increased fetal susceptibility to the effects of toluene on skeletal development
da-Silva *et al*. (1990b)	Wistar rats (n=11-13) and hamsters (n=10)	rats: GD 14-20 hamsters: GD 6-11 sacrificed?	0, 800 mg/m^3 6h/d by inhalation	no maternal toxic effects observed	in rats, the number of pups born alive was decreased and number of litters with low birth weight was increased. Male rat offspring exposed to toluene displayed shorter latencies in spontaneous alternation test and exposed hamsters performed worse in a rotating rod test than controls (only on 1 test day).	

n= number of animals per group; PN= post-natal day; GD= gestation day; h= hours; d= day; bw= body weight

Table 2.4 Development toxicity studies in animals with toluene.

authors	species	experimental period/design	dose and route	general toxicity	developmental toxicity	remarks
Klimisch *et al*. (1992)	Himalayan rabbits exp.1) n=15 exp.2) n=20	GD 6-18 sacrifice: GD 29	1) 0, 113, 375 and 1125 mg/m^3 6h/d 2) 0, 375, 1875 mg/m^3 6h/d by inhalation	no maternal toxicity observed	no effect on reproduction, no effect on fetal retardations, variations and malformations except for incidence of skelatal retardations in experiment 1 which was increased	
Gospe *et al*. (1994)	Sprague-Dawley rats (n=11)	GD 6-19 sacrifice: GD 19	520 mg/kg bw by gavage	decreased bw	decreased fetal body weight, placenta weight and several organ weights. No effect on number of implantations, stilbirths, congenital malformations and neuropathological changes.	
Ono *et al*. (1995)	Sprague-Dawley rats (n=20)	GD 7-17 sacrifice: GD 20(n=13), the others were allowed to litter	0,2250, 7500 mg/m^3 6h/d by inhalation	*2250 mg/m^3* : no toxic effects *7500 mg/m^3*: maternal bw and fc were decreased during exposure period	*2250 mg/m^3* : no toxic or teratogenic effects *7500 mg/m^3* : fetal body weights decreased, fetal mortality increased and fetal growth retarded. No effect on external, internal and skeletal anomalies and no effect on pre- and postweaning behavioural tests.	
Gospe *et al.* (1996)	Sprague-Dawley rats (n=8)	GD 6-19 sacrifice: GD 19	0, 650 mg/kg bw by gavage	maternal bw and fc decreased	foetal body weights and weights of several organs decreased. Skeletal ossification delayed. Morphometric analysis of brain sections revealed several effects.	

n= number of animals per group; bw= body weight; GD= gestation day; d= day; h-hour; fc-food consumption; wc= water consumption

Table 2.5 Development toxicity studies in animals with toluene.

authors	species	experimental period/design	dose and route	general toxicity	developmental toxicity	remarks
Jones and Balster (1997)	CD-1 mice (n=13)	GD 12-17 sacrificed?	0, 750, 1500, and 7500 mg/m^3. 3 times a day for 60 min by inhalation	no maternal toxicity observed	*750, 1500 mg/m^3* : no effects of toluene *7500 mg/m^3* : no effects on gestational lenght, number of litters and litter size. Decreased performance in various behavioural test performed between PN 1-20	
Thiel and Chahoud (1997)	Wistar rat 1) n=38 2) n= 23 3) n=23 4) n=29 5) n=24	GD 9-21 sacrificed ?	1) 0 mg/m^3 6h/d 2) 1125 mg/m^3 6h/d 3) 2500 mg/m^3 6h/d 4) 3750 mg/m^3 6h/d 5) 4500 mg/m^3 6h/d by inhalation	4500 mg/m^3 : maternal bw decreased.	*4500 mg/m^3* : body weights of offspring reduced, increased pup mortality until weaning, retarded physical development No effects on development and learning ability, no effects on mating, fertility and pregnancy indexes.	
Gospe *et al.* (1998)	Sprague Dawley rats (n=18)	GD 6-19 sacrifice: GD 19 (n=5), PN 10 (n=5), PN 21 (n=5)	0 and 650 mg/kg bw by gavage	maternal toxicity not presented	growth retardation (body and organ weights and brain morphometry) on GD19 and PN10. Most effects were resolved on PN21.	
Hass *et al.* 1999	Wistar rats (control n=14; exposed n=18)	GD 6 untill day 18 postnatally	0 and 4500 mg/m^3 (0 and 1200 ppm) 6 hours/day by inhalation	decreased maternal weight	decreased fetal weight (weight 12% of the control weight), delayed ontogeny of the reflexes, increased motor activity in the open field of male and female offspring. impaired congenitive function at the age of 3.5 months	
Hougaard *et al.* (1999)	Wistar rat (n=16)	GD 7-20 sacrificed ?	0, 6750 mg/m^3 6h/d by inhalation	slight, not significant decreased bw	decreased pup bw no effects motor function, activity level, acoustic startle and prepulse inhibition. Small effects in hearing function of male pups and impaired performance in Morris water maze of female pups.	

n= number of animals per group; bw= body weight; GD= gestation day; d= day; h-hour; fc-food consumption; wc= water consumption

Annex **E**

Calculation safe levels of toluene 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 toluene as an adult.

The RIVM (Jan94) proposed an ADI of 430 µg/kg body weight/day.
Maximal permissible level per infant is 1935 µg/infant/day.
Maximal permissible level of toluene in breast milk is 2150 µg/l = ~ 2.15 mg/l.

In conclusion, the committee considers 2.2 mg toluene/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