Styrene

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp	: Aanbieding advies over styreen
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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 styreen. 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

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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

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 styreen 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 styreen niet te classificeren.
- Voor effecten op de ontwikkeling, meent de commissie dat er onvoldoende geschikte gegevens zijn. Zij adviseert daarom om styreen niet te classificeren.
- Voor effecten tijdens lactatie, adviseert de commissie om styreen 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 styrene.

The committee's recommendations are
- For effects on fertility, the committee recommends no classification of styrene due to a lack of appropriate data.
- For developmental toxicity, the committee recommends not classify styrene due to a lack of appropriate data.
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data styrene should not be labelled.

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 re-
quested the Health Council of the Netherlands to classify compounds toxic to reproduc-
tion. 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 Mini-
stry of Social Affairs and Employment to extend the existing list of compounds classi-
fied 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 styrene 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 Reproduc-
tion 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 will 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).

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* 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 to 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. The quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered for each study.

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.

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*   for definitions see Tox95

Styrene

2.1 Introduction

Name	:	styrene
CAS reg no	:	100-42-5
Synonyms	:	Vinylbenzene, ethylenebenzene, cinnamene, phenylethylene
Examples of use	:	Styrene is used as an organic solvent and as a cross linking agent in the reinforced plastic industry. glue and rubber industry.
Mol weight	:	104.16
Chem formula	:	C_8H_8
Conversion factor	:	1 ppm = 4.33 mg/m^3 at 760 mm Hg and 20 ^{o}C
		1 mg/m^3 = 0.23 ppm

2.2 Human studies

Fertility

Härkönen and Holmberg (Har82) examined the duration of the menstruation and the incidence of irregular menstruation in 67 Finnish female lamination workers and their matched controls. No differences between exposed and controls were observed. During the styrene exposure period there was no effect of styrene on the incidence of sponta-

neous abortions. Most probably, the female workers were exposed to a mixture of organic compounds.

Serum levels of prolactin, growth, thyroid-stimulating, gonadotrophin follicle-stimulating and luteinizing hormones were measured in 30 females exposed to about 130 ppm (= 563 mg/m^3) styrene (range 280-1300 mg/m^3) and in 30 controls (Mut84). Prolactin and growth hormone serum levels were statistically significantly increased in the exposed women. No effect of styrene exposure was observed on serum levels of the other hormones measured.

Lemasters *et al.* (Lem85) studied 174 exposed and 449 unexposed female workers from 36 USA companies that made reinforced plastic. No effects of exposure on menstrual disorders were detected. The female workers were most probably exposed to styrene and other organic solvents.

Jelnes (Jel88) described semen quality in workers from a Danish factory that produces reinforced plastics. The air in the plant was found to contain styrene and acetone. Workplace median styrene concentrations had been 68, 84 and 128 ppm, while simultaneously obtained acetone levels were 0.3 and 0.6 ppm (median values) (Danish TLVs 25 ppm and 250 ppm, respectively). The workers were also exposed to heat. Semen and blood samples were collected from 25 men within 3 weeks after production at the plant had stopped. Men providing their first semen sample at the fertility clinic were chosen as a reference group. The two groups did not differ in serum LH, serum FSH, semen volume or sperm count. Sperm of the exposed group showed a higher percentage of live and motile cells. In contrast, the percentage of normal sperm was lower in the exposed group. However, the choice of the control group may mask the effects in the exposed group and the workers were exposed to various organic solvents and heat.

Brown (1991) mentioned that menstrual irregularities were reported by several Russian authors but that it is difficult to assess the overall significance of these studies because they suffer in a lack of presentation, poor data, lack of appropriate controls and analysis (Bro91).

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 correlated 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 styrene were 0.59 (95% CI 0.25-1.39) (low exposure, n=6) and 1.00 (95% CI 052-1.93) (high exposure, n=12).

Preliminary results of the European Asclepios project on occupational hazards to male reproductive capability showed no detrimental effect of styrene exposure on male fecundity (Kol99a) and although a declining sperm count was suggested following styrene exposure there was no exposure-response relationship between styrene exposure and semen quality (Kol99b,c). Workers were most probably exposed to styrene and other organic solvents.

Development

Holmberg (Hol79 and Hol80) identified 132 cases of children with congenital central nervous system defects for a two-year period in material of the Finnish Register of Congenital Malformations (both articles described the same population). One hundred twenty of them and their controls 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 controls).

In Finnish studies (Hem80 and Hem84) there were higher rates of spontaneous abortions among female chemical workers occupationally exposed to styrene. However, on extending the follow-up period an increase in spontaneous abortion rates in styrene exposed workers was not confirmed. Female workers were most probably exposed to styrene and other organic solvents.

In a matched case-control study of Lindbohm *et al.* (Lin85), the relationship between the risk of spontaneous abortions and the occupational exposure to compounds in the plastic industry was investigated. Information on spontaneous abortions (cases, n=44) and births (controls, n=123) was obtained from the hospital discharge register whereas data on occupational exposures were obtained from the occupational health services of the workplaces. No increased risk of spontaneous abortions was observed

among workers processing polymerized plastics or heated plastics made of vinyl chloride or of styrene. Female workers were most probably exposed to mixtures of organic solvents including styrene.

McDonald *et al.* (Don88) used the data from a large survey in Montreal from 1982 to 1984 to study spontaneous abortions in women working in plastic factories. In this study there were 26 pregnancies and 5 spontaneous abortions designated "polystyrene work alone" and 50 pregnancies and 13 spontaneous abortions involving "mixed including polystyrene" exposure. After correction for the confounding factors the observed to expected spontaneous abortion ratio for women processing polystyrene was 1.58 (90% CI 1.02-2.35).

In a study of Lemasters *et al.* (Lem89), the birth weight data of infants whose mothers (n=1535) worked in the reinforced plastics industry during pregnancy were analysed. There was no significant dose-response effect, however, women with the most highly exposed jobs (estimated mean styrene exposure of 82 ppm = 355 mg/m^3) had offspring with adjusted birth weight of 4% lower than the offspring of unexposed women. Women in these jobs were exposed to mixtures of organic solvents.

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 styrene exposure was 0.3 (95% CI 0.1-1.0) (3 cases and 17 controls).

Lactation

Fisher *et al.* (Fis97) studied the human blood/air and milk/air partition coefficients (PC) in human blood and human milk samples. The objective of this study was to evaluate the potential chemical exposure of nursing infants that ingested contaminated milk from mothers who were 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 styrene (50 ppm = 217 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 styrene ingested by a nursing infant over a 24-hour period was 0.65 mg in 0.92 l (0.71 mg/l).

Based on the proposed ADI of 77 µg/kg body weight/day (RIVM: Jan94), a maximal permissible level of about 0.4 mg/l breast milk can be calculated for styrene (see Annex E).

2.3 Animal studies

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

Fertility

Beliles *et al.* (Bel85) evaluated the reproductive performance in groups of Sprague-Dawley (COBS(SD)BR) rats that received styrene in their drinking water at concentrations of 0, 125 and 250 ppm (= 0, 125, 250 mg/l; estimated daily intake of 14 and 21 mg/kg body weight/day for the high dose males and females, respectively). Due to the limited solubility in drinking water the dosages used in this study were rather low. No treatment-related findings were observed. Approximately 100 days after the start of exposure, 10 males and 20 females were mated to produce F1 pups. These pups were subsequently mated to produce 3 generations of offspring, all maintained on styrene-treated drinking water. A loss of breeding efficiency was observed in the F3 parents; however, this effect could be attributed to 1 male. No other effects on reproduction and fertility were noted (for a more detailed description see section developmental toxicity).

Srivastava *et al.* (Sri89) studied the effects of administration of styrene (0, 200, and 400 mg/kg body weight by gavage) to adult male Wistar rats for 60 days. No effects on body weight, testes or epididymis weight were observed. No overt signs of testicular toxicity were noted at the lower dose, while some marker enzymes for testicular function were found to be altered significantly, along with a decrease in the number of spermatozoa at the high dose. In the high dose group histopathological examination revealed marked degeneration of seminiferous tubules and lumen devoid of sperm. Other toxic effects were not described. Only six animals per group were tested.

Srivastava *et al.* (Sri92a) studied the toxic effects (on testicular enzymes) on the unweaned pups of lactating dams that received gavage doses of 0, 100 and 200 mg styrene/kg body weight by gavage) during postnatal days 1 to 60. A significant decrease in epididymal spermatozoa count and testis weight was observed at the high dose. Activities of testicular sorbitol dehydrogenase and acid phosphatase decreased, while activities of lactate dehydrogenase, β-glucuronidase, glucose-6-phosphate dehydrogenase and γ-glutamyl transpeptidase significantly increased in the high dose group. Other effects were not described.

Waalkens-Berendsen (1999a) exposed male Wistar rats by gavage to 0 or 400 mg styrene/ kg body weight /day for 28 days (Waa99). Animals were sacrificed 1, 7 and 28 days after the last day of administration. At sacrifice liver and reproductive organs were weighed and preserved for further microscopic examination. In addition, epididymal sperm motility, count and morphology and testicular sperm count were performed. During the first week of treatment a statistically significant reduction in growth was observed in the styrene treated animals. Relative liver weight was increased at sacrifice 1 day after the last treatment. Epididymal sperm count was statistically significantly decreased in the animals sacrificed 28 days after the last treatment with styrene but no effect was observed in the animals sacrificed 1 or 14 days after the last treatment. Furthermore, since no effect was observed on other sperm parameters, microscopic examination or weight of the reproductive organs, the biological significance of the effect on epididymal sperm count is unclear.

Development

Murray *et al.* (Mur78) exposed mated Sprague-Dawley rats and New Zealand White rabbits by inhalation to 0, 300 and 600 ppm styrene (0, 1299, 2598 mg/m^3) 7 hours/day from gestation days 6 through 15 (rats) and 6-18 (rabbits). Additional groups of rats were given styrene by gavage at dose levels of 0, 90 or 150 mg/kg bw twice daily (0, 180 or 300 mg/kg/day, respectively) from gestation days 6 through 15 of gestation. Embryotoxicity and foetotoxicity were not evident in rats or rabbits inhaling styrene or in rats given styrene orally. Maternal effects, decreased body weight gain and decreased food consumption (only stated no data given), were noted in all groups of rats given styrene but were not observed in rabbits. The report states that the incidences of skeletal variants in the rat was significantly higher in styrene litters than controls; but within historical ranges, no data were presented. For the rabbits the report states that the incidence of minor skeletal variants was within the range normally observed among control groups from other recent studies. No teratogenic effect was detected in either species inhaling styrene or in rats given styrene by gavage.

Kankaanpää *et al.* (Kan80) studied the effects of inhaled styrene exposure in mice and Chinese hamsters. Mice (BMR/T6T6) were exposed to 0 and 250 ppm (0, 1083 mg/m3) for 6 hours/day from gestation days 6 to 16 and sacrificed on day 16. Maternal toxic effects were not described. The number of dead or resorbed foetuses and the number of malformed foetuses were slightly increased in the exposed group. In a preliminary study mice were exposed to 500 and 750 ppm (2165, 3248 mg/m^3). In the 500 ppm group, 2 of 6 mice died and in the 750 ppm group 3 of 5 exposed mice died. The foetal death rate in surviving dams was 47 and 95% in the 500 and 750 ppm groups, respectively. Chinese hamsters were exposed to 0, 300, 500, 750 and 1000 ppm styrene

(0, 1299, 2165, 3248, 4330 mg/m^3) for 6 hours/day from gestation days 6 to 18 (in this study day 1 was the first day of pregnancy). No effects on developmental parameters were observed in the 300 to 750 ppm group. The number of dead and resorbed foetuses was statistically significantly increased in the 1000 ppm group. Maternal toxicity was not reported.

Beliles *et al.* (Bel85) evaluated the reproductive performance in groups of COBS (SD)BR rats receiving styrene in their drinking water at concentrations of 0, 125 and 250 ppm (estimated daily intake 14 and 21 mg/kg body weight/day, respectively at the high dose for the males and females). Due to the limited solubility in drinking water the dosages used in this study were rather low. No consistent toxic effects of styrene treatment were observed. Approximately 100 days after the start of the exposure period, 10 males and 20 females were mated to produce F1 pups. These pups were subsequently mated to produce 3 generations of offspring, all maintained on styrene-treated drinking water. For each generation, the following were evaluated: fertility, litter size, pup viability, pup survival, sex ratio, pup body weight and weanling liver and kidney weight. Some significant effects on pup survival in the first and second generation was observed in the high dose group, however, this effect could be attributed to only two litters.

In a study of Srivastava *et al.* (Sri90) pregnant Wistar rats were treated with styrene by gavage at dose levels of 0, 250 and 400 mg/kg styrene from day 6-15 of gestation. Maternal body weights in the 400 mg/kg group were statistically significantly decreased. In this group, a decreased number of implantations was observed, although the exposure started at day 6 of gestation and was continued up to day 15 of gestation. Furthermore, in the 400 mg/kg group, the number of dead and resorbed foetuses was increased and foetal weight was decreased. No skeletal malformations were observed.

Pregnant Sprague-Dawley rats were given 0 or 300 mg/kg styrene by gavage on GD 11 and sacrificed on GD 12 (for hepatic metallothionein analysis or Zn distribution to maternal or embryonic tissues) or on GD 20 (to assess the developmental effects of styrene exposure) (Das91). Food consumption and body weights were statistically significantly decreased in the treated dams. No developmental toxicity was observed in litters of styrene-treated dams. Hepatic metallothionein concentration was increased by styrene which could be attributed to a decreased food consumption since similar effects were observed in a pair-fed group. Styrene treatment had no effect on Zn distribution.

Pregnant Wistar rats were exposed via inhalation to 0, 50 or 300 ppm (0, 217, 1299 mg/m^3) styrene during GD 7-21 for 6h/day and the offspring were subsequently evaluated in several neurobehavioural tests (Kis95). Except for a not statistically significant decrease in body weights of the dams of the highest dose group, styrene treatment induced neither maternal toxicity nor adverse foetal developmental effects. At 21 and 77 days of age but not at 125 days body weights of the pups of the highest dose group were decreased. Preliminary results with a small number of litters (5, 2 and 5 litters in the

control, 50 and 300 ppm groups, respectively) revealed significant dose-dependent effects in tests performed prior to weaning (surface righting, pivoting locomotion and bar holding) as well as in tests performed after weaning (motor co-ordination, open-field behaviour and motor activity)

Several neurochemical effects in the developing brain of the offspring of Wistar rats exposed by inhalation to 50 or 300 ppm styrene (217 or 1300 mg/m^3) from GD 6-20 for 6 hours a day were observed by Katakura *et al.* (Kat99). Data on maternal toxicity were not presented. Food intake in the 300 ppm was decreased. A restricted pair-fed control group was included in the study. Most effects were not statistically significant compared to this group.

Mated female rats were treated daily by gavage with 0, 50, 100 or 200 mg styrene/kg body weight from GD 6 to PN day 14 (Waa99b). Randomly selected pups were sacrificed on PN days 4 and 21; liver, spleen, thymus, testis, epididymis, ovaries and uterus weights were recorded. Furthermore, sexual maturation was followed and oestrus cycle of the F1-females was evaluated. F1-males and females of the 200 mg/kg were mated with control F1-animals. The pregnant females were sacrificed on GD 11 and the number of corpora lutea were counted and the uterus was examined for the number of implantations, dead embryos and resorptions. At sacrifice (age about 16 weeks) the reproductive organs of the F1-males were weighed and the sperm parameters (epididymal sperm motility, count and morphology and testicular sperm count) were determined. In the F0-females no effect on reproduction was observed. In the 100 and 200 mg/kg group the body weight was significant decreased when compared to the control group. The number of pups/litter, number of dead pups, litter and pup weight and sex ratio were comparable between the groups. No effect on organ weight or on histopathological findings were observed in the F1-pups on PN days 4 and 21. No effect was observed on sexual maturation, oestrus cycle or reproduction capacity of the F1-animals. Neither microscopic nor sperm parameters revealed any styrene induced effects.

Lactation

In a study of Srivastava *et al.* (Sri92b) female rats were orally treated with 0, 200 and 400 mg styrene/kg during postnatal days 0-21. On PN days 31, 61 and 91, six pups per group were sacrificed for determination of testicular enzyme activities, number of epididymal spermatozoa and testicular pathology. Activities of various enzymes were altered in the 400 mg/kg group at PN days 31 and 61 but not at PN day 91. Furthermore, in this group the number of epididymal spermatozoa was statistically significantly decreased at PN day 61 and 91. No effect was observed on testicular histopathology.

2.4   Conclusion

In humans, no effect of occupational exposure to styrene was observed on the incidences of menstrual disorders (Här82 and Lem85). Jelnes *et al.* (Jel88) observed an effect on the incidence of abnormal sperm after occupational exposure. However, the workers were also exposed to other organic solvents (acetone) and the choice of the control group in this study may mask a possible effect. No effect of occupational styrene exposure was observed in a time-to-pregnancy study of Sallmén *et al* (Sal95). Preliminary results of the European Asclepios project showed no clear effects of styrene exposure on male fecundity and semen quality (Kol99a, Kol99b, Kol99c).

In a 3 generation reproduction study of Beliles *et al.* (Bel85), no effects on fertility and reproduction of rats were found at relative low doses of styrene in the drinking water (up to 250 ppm, corresponding to 14 mg/kg body weight and 21 mg/kg body weight in males and females, respectively). A study with styrene at high dosages, 200 and 400 mg/kg bw, in young and adult rats, respectively, suggested an effect on male fertility (Sri89 and Sri92a). However, more data should be provided on the reproductive performance and fertility to verify these effects. In a rats study by Waalkens-Berendsen (Waa99), no significant effects on sperm parameters, organ weights and histopathology of the reproductive organs were observed after 28 day oral exposure to styrene.

In view of the human and animal data, the committee recommends not to classify styrene for effects on fertility because a lack of appropriate data.

Initial human studies of Holmberg (Hol79) suggested an association of styrene exposure with the incidences of CNS malformations and spontaneous abortion. However, more extended studies of investigators of the same group (Hol82 and Kur83) disproved these suggestions. In studies of Hemminki *et al.* (Hem80 and Hem84), Lindbohm *et al.* (Lin85 and Lin90) and McDonald *et al.* (Don88) no effect after occupational exposure to styrene was observed on the incidences of spontaneous abortions. Lemasters *et al.* (Lem89) showed that women exposed to high concentrations of styrene (355 mg/m^3) gave birth to children with a slightly lower birth weight. However, these women were also exposed to other solvents.

The developmental toxicity of styrene was tested in several mammalian species: rat, mouse, rabbit and Chinese hamster. In a study of Murray *et al.* (Mur78) rats were treated by inhalation or by gavage and rabbits by inhalation. Although maternal toxicity was observed, no embryotoxic, foetotoxic or teratogenic effects were observed. In a study of Kankaanpää *et al* (Kan80), developmental effects were observed in mice at maternally toxic dose levels (mice). In Chinese hamsters the number of dead or resorbed foetuses was increased after exposure to 1000 ppm styrene. However, in this group only 7

litters were studied. In a two-generation study of Beliles *et al.* (Bel85), significant adverse effects on pup survival were observed; However, these effects could be attributed to only two litters. Srivastava *et al.* (Sri90) observed an increased number of dead and resorbed foetuses and a decreased foetal weight in rats treated with 400 mg/kg styrene by gavage from gestation day 6 to 15. No teratogenic effects were observed. The decreased maternal body weight was probably caused by the decreased number and the retarded growth of the foetuses. In the study of Daston *et al.* (Das91), no developmental effects were observed in rats receiving styrene by gavage at maternally toxic doses. Preliminary results of a study of Kishi *et al.* (Kis95) with rats exposed to styrene by inhalation on GD 7-21 revealed some neurobehavioural effects in the offspring. However, in this study only a small number of litters were used. Slight neurochemical effects in the developing brain of the offspring of rats were observed by Katakura *et al.* (Kat99).

Waalkens (1999) studied the developmental toxicity of styrene in rats; styrene was dosed from GD 6 to PN day 14. No effects on reproduction and development of the F0- and F1-animals were observed.

In conclusion, in view of the human and animal data, the committee recommends not to classify styrene for effects on development because a lack of appropriate data.

From the study of Fisher *et al.* (a pharmacokinetic lactation model), an amount of 0.71 mg styrene/l breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) styrene 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 styrene in human breast milk and about the possible effects during lactation.

In the study of Srivastava *et al.* (Sri92b) testicular enzyme activities and the number of epididymal spermatozoa in the male offspring of rats exposed to styrene during lactation were affected. However, in this study data about styrene concentrations in milk are missing.

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

Proposed classification for fertility

Lack of appropriate animal and human data precludes assessment of styrene for effects on fertility

Proposed classification for developmental toxicity

Lack of appropriate animal and human data precludes assessment of styrene for effects on development.

Proposed labelling for effect during lactation

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

Additional consideration

The committee would like to emphasize that several human studies considered here in view of styrene 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 styrene or a mixture of solvents containing styrene. Therefore, the EU Classification and Labelling guideline does not warrant a classification of styrene 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 styrene.

References

Bel85   Beliles RP, Butala JH, Stack CR, Makris S. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fund. Appl. Toxicol. 1985; 5: 855-868.

Bro91   Brown NA. Reproductive and developmental toxicity of styrene. Reprod. Toxicol.1991; 5: 3-29.

Das91   Daston GP, Overmann GJ, Taubeneck MW, Lehman-McKeeman LD, Rogers JM, Keen CL. The role of methallothionein induction and altered zinc status in maternally mediated developmental toxicity: comparison of the effects of urethane and styrene in rats. Toxicol. Appl Pharmacol. 1991; 110: 450-463.

Don88   McDonald AD, Lavoie J, Cote R, McDonald JC. Spontaneous abortion in women employed in plastics manufacture. Am. J. Indust. Med. 1988; 14: 9-14.

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.

Har82   Härkönen H, Holmberg PC. Obstetric histories of women occupationally exposed to styrene. Scand. J. Work. Environ. Health 1982; 8: 74-77.

Hem80   Hemminki K, Fransilla E, Vainio H. Spontaneous abortions among female chemical workers in Finland. Int. Arch. Occup. Environ. Health 1980; 45: 123-126.

Hem84   Hemminki K. Reproductive hazards and plastic industry. In: Industrial Hazards of Plastics and synthetic Elastomers"" 1984: 79-87.

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

Hol82   Holmberg PC, Hernberg S, Kurppa K *et al*. Oral clefts and organic solvent exposure during pregnancy. Int. Arch. Occup. Environ. Health 1982; 50: 371-376.

Jan94   Janus JA, Hesse JM, Rikken MGS (eds.) Aandachtstoffen in het nederlandse milieubeleid - overzicht 1994. RIVM rapport nr. 601014006 november 1994.

Jel88   Jelnes JE. Semen quality in workers producing reinforced plastics. Reprod. Toxicol. 1988; 2: 209-212.

Kan80   Kankaanpää JTL, Elovaara E, Hemminiki K, Vainio H. The effect of maternally inhaled styrene on embryonal and fetal development in mice and Chinese hamsters. Acta Pharmacol. Toxicol. 1980; 47: 127-129.

Kat99   Katakura Y, Kishi R, Ikeda T, Miyake H. Effects of prenatal exposure to styrene on neurochemical levels in rat brain. Toxicol. Lett. 1999; 105: 239-249.

Kis95   Kishi R, Qing Chen B, Katakura Y, Ikeda T, Miyake H. Effects of prenatal exposure to styrene on the neurobehavioral development, activity, motor coordination and learning behavior of rats. Neurotoxicol. Teratol. 1995; 17: 121-130.

Kol99a   Kolstad HA, Bisanti L, Roeleveld N *et al.* Timet o pregnancy for men occupationally exposed to styrene in several European reinforced plastics companies. Scand. J. Work Environ. Health. 1999; 25 suppl: 66-69

Kol99b   Kolstad HA, Bonde JPE, Spano M *et al.* Sperm chromatin structure and semen quality following occupational styrene exposure. Scand. J. Work Environ. Health. 1999; 25 suppl: 70-73.

Kol99c   Kolstad HA, Bonde JP, Spano M *et al.* Change in semen quality and sperm chromatin structure following occupational styrene exposure. Int. Arch Occup. Environ. Health 1999; 72: 135-141.

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

Lem85   Lemasters GK, Hagen A, Samuels SJ. Reproductive outcomes in women exposed to solvents in 36 reinforced plastic companies; I.Menstrual dysfunction. J. Occup. Med. 1985; 27: 490-494.

Lem89   Lemasters GK, Samuels SJ, Morrison JA, Brooks SM. Reproductive outcome of pregnant workers employed at 36 reinforced plastics companies. II. Lowered birth weight. J. Occup. Med. 1989; 2: 115-120.

Lin85   Lindbohm M-L. Hemminki K, Kyyrönen P. Spontaneous abortions among women employed in the plastic industry. Am. J. Ind. Med. 1985; 8: 579-586.

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.

Mur78   Murray FJ, John JA, Balmer MF, Schwetz BA. Teratologic evaluation of styrene given to rats and rabbits by inhalation and by gavage. Toxicol. 1978; 11: 335-343.

Mut84   Mutti A, Vescovi PP, Falzoi M, Arfini G. Neuroendocrine effects of styrene on occupationally exposed workers. Scand. J. Work. Health 1984; 10: 225-228.

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.

Sri89   Srivastava S, Seth PK, Srivastava SP. Effect of styrene administration on rat testis.Arch. Toxicol. 1989; 63: 43-46.

Sri90   Srivastava S, Srivastava SP, Seth PK. Embryo/Fetotoxicity of styrene in rats. J. Environ. Biol. 1990; 11: 73-77.

Sri92a   Srivastava S, Seth PK, Srivastava SP. Effect of styrene on testicular enzymes in growing rat. Ind. J. Exp. Biol. 1992; 30: 399-401.

Sri92b   Srivastava S, Seth PK, Srivastava SP. Biochemical and morphological studies in testes of rat offspring of mothers exposed to styrene during lactation. Pharmacol. Toxicol. 1992; 70: 314-316.

References   25

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

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

Waa99a   Waalkens-Berendsen DH. Testicular effects of styrene in rats. TNO study P1861.

Waa99b   Waalkens-Berendsen DH. Oral reproduction study with styrene in rats. TNO study P1860.

Literature consulted but not cited

Age92   Agency for Toxic Substances and Disease Registry. Toxicological profile for styrene. 1992

Ahl87   Ahlborg G Jr, Bjerkedal T, Egenaes J. Delivery outcome among women employed in the plastics industry in Sweden and Norway. Prog. Clin. Biol. Res. 1987; 12: 507-517.

And80   Andersson HC, Tranberg EA, Uggla AH, Zetterberg G. Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory. Mut. Res. 1980; 73: 387-401.

Bar82   Barlow SM, Sullivan FM. Reproductive hazards of industrial chemicals. An evaluation of animal and human data. Acad. Press Inc. (London), 1982: 501-514.

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. Reprod. Toxicol. 1994; 8: 121-135.

Che90   Chernoff N, Setzer WR, Miller DB, Rosen MB, Rogers JM. Effects of chemically induced maternal toxicity on prenatal development in the rat. Teratol. 1990; 42: 651-658.

Els83   Elskamp DMW. Toxicologie van styreen. Medisch Laboratorium TNO rapport. Rapport no. MBL 1983-11. 1983.

Har84   Härkönen H, Tola S, Korkala ML, Hernberg S. Congenital malformations, mortality and styrene exposure. Ann. Acad. Med. Singapore 1984; 13: 404-407.

Hol77   Holmberg PC. Central nervous defects in two children of mothers exposed to chemicals in the reinforced plastics industry. Scand. J. Work. Environ. Health 1977; 3: 212-214.

Ike82   Ikeda M. The toxicological evaluation of styrene as an industrial chemical. Jpn. J. Ind. Health 1982; 24: 581-598. (Abstract, article in Japanese).

Izy72   Izyumova AS. The action of small concentrations of styrol on the sexual function of albino rats. Gigiena I. Sanitariya 1972; 37: 29-30 (article in Russian with English abstract)

Joh78   Teratologic evaluation of vinylchloride, vinylidene chloride and styrene in laboratory animals. Teratology 1978; 17: 48A (abstract)

Kan79   Kankaanpää JTJ, Hemminki K, Vainio H. Embryotoxicity and teratogenicity of styrene and styrene oxide on chick embryos enhanced by trichloropropylene oxide. Acta Pharmacol. et Toxicol. 1979; 45: 399-402.

Kis92   Kishi R, Katakura Y, Ikeda T et al. Neurochemical effects in rats following gestational exposure to styrene. Toxicol. Letters 1992; 63: 141-146.

Lin93   Lindbohm M-L. Effects of styrene on the reproductive health of women: a review. In: Butadiene and styrene: Assessment of Health Hazards. Eds. Sorsa M, Peltonen K, VainioH,Hemminki K. IARC Scientific Publications No. 127. 1993: 163-169.

Lin99   Lindbohm M-L. Effects of occupational solvent exposure on fertility. Scand. J. Work Environ. Health 1999; 25 Suppl: 44-46.

Pon78   Ponomarkov V, Tomatis L. Effects of long-term oral administration of styrene to mice and rats. Scand. J. Work Environ. Health 1978; 4: 127-135.

Rag74   Ragul'ye N. The problem of the embryotoxic action of styrol. Gig. Sanit. 1974: 85-86 (article in Russian)

Roe94   Roe FJC. Styrene: Toxicity studies - what do they show? Crit. Rev. Toxicol. 1994; 24: S117-S125

Sal85   Salomaa S, Donner M, Norppa H. Inactivity of styrene in the mouse sperm morphology test. Toxicol. Lett 1985, 24, 151-155

Sch93   Schardein JL. Chemically induced birth defects. 2nd ed., rev. and expanded Marcel Dekker, Inc., New York, 1993: 787-799.

Sha84   Shanker J, Prasad ARK, Datta K. Embryotoxicity of styrene and its effect on heme biosynthesis. Indian J. Exp. Biol. 1984; 22: 167-168.

She89   Shepard TH. Catalog of teratogenic agents, 7th edition. John Hopkins University Press, Baltimore MD, 1989: 585.

Vai77   Vainio H, Hemminki K, Elovaara E. Toxicity of styrene and styrene oxide on chick embryos. Toxicol 1977; 8: 319-325.

Ver79   Vergieva T, Zaikov Kh, Platov S. Study of the embryotoxic action of styrene. Khig. Zdraveopaz 1979; 22: 39-43.

WHO83   World Health Organization. Styrene. Environmental Health Criteria 1983; 26.

WVD89   Rapport inzake grenswaarde styreen: gezondheidskundig advies van de Werkgroep van Deskundigen ter vaststelling van MAC-waarden. 1989

Zai85   Zaidi NF, Agarwal AK, Srivastava SP, Seth PK. Effect of gestational and neonatal styrene exposure on dopamine receptors. Neurobeh. Toxicol. Teratol. 1985; 7: 23-28

References   27

Annexes

A

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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:

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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 interfe-

re 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

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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.

D

Fertility and developmental toxicity studies

See next pages.

Table 1 Fertility studies with styrene.

authors	species	route	experimental period	dose	findings	remarks
Belilies *et al.* (1985)	COBS (SD)BR rats (control: 76 male and 106 females styrene: 50 male and 70 females)	drink	prem (100 d), mating, gestation and lactation during 3 generations	0,125 and 250 ppm (0,541 and 1083 mg/m3)	*125 ppm*: no effects *250 ppm*: F1-pups: day 21 survival index decreased*, F2-pups: reduced pup survival days 1, 7, and 14, F2-females: reduced number of females which produced litters**, F3-pups: no effect on survival	* all dead pup were in two litters ** 2 of 5 females that failed to litter were mated to the same male, when corrected for this male the values were comparable with the controls
Srivastava *et al.* (1989)	Wistar rats males (n=6)	gav	60 days	0,200 and 400 mg/kg bw	*400 mg*: no effects on body weight, testis and epididymides weight, alterations testicular enzymes and degeneration seminiferous tabules and lumina devoid of sperm	other toxic effects not decribed, data for body weight and organ weight not presented; only six animals per group were tested
Srivastava *et al.* (1992a)	Wistar rats males (n=21)	gav	PN days 1-60	0, 100 and 200 mg/kg bw	*200 mg/kg bw*: decreased epididymal sperm, decreased tetis weight, decreased activities of testicular sorbitol dehydrogenase and acid phosphatse decreased, increased activities of lactate dedydrogenase, ß-glucuronidase, glucose-6-phosphate dehydrogenase and γ-glutamyl transpeptidase	other toxic effects were not decribed
Waalkens (1999a)	Wistar rats males (n=6/group per sacrifice day	gav	daily for 28 days, Sacrifice days 29, 35 and 56	0 or 400 mg/kg bw	decreased bw change and food consumption day 0-7 At sacrifice at day 29: increased relative liver weight. No effects on organ weight and microscopy and sperm parameters. At sacrifice at day 35: No effects on organ weight and microscopy and sperm parameters At sacrifice at day 56: decreased epididymal sperm count no other effects on organ weight and microscopy and sperm parameters	

bw = body weight; drink= drinking water gav= gavage prem= premating; PN= postnatal; n=number of animals per group

Table 2.1 Developmental toxicity studies with styrene.

authors	species	route	Experimental period	dose	findings	remarks
Kankaanpää *et al.* (1980)	Mice BMR/T6T6 (n=13-15)	inh	gestation days 6-16, sacrifice day 16	0 and 250 ppm 6h/day (0, 1083 mg/m^3)	*250 ppm*: increased no. resorptions, 3 malformed foetuses	no foetal visceral examination. No appropriate statistics
Kankaapää *et al.* (1980)	Chinese hamster (n=2-15)	inh	gestation days 6-18, sacrifice day 18	0, 300, 500, 750 and 1000 ppm 6h/day (0, 1299, 2165, 3248, 4330 mg/m^3)	*1000 ppm*: increased no, of resorptions, no malformations	number of animals per group very low 15, 2, 3, 5 and 7 for the control 300, 500, 750 and 1000 ppm group, respectively. Maternal toxicity inadequately reported
Murray *et al.* (1978)	Sprague Dawley rat (n=29-30)	inh	gestation days 6-15, sacrifice day 21	0, 300 and 600 ppm 7h/day (0,1299, 2598, mg/m^3)	*300 ppm*: maternal toxicity; decreased bw gain days 6-9, no embryo-or foetal toxic effects, increased incidence of minor skeletal variants *600 ppm*: maternal toxicity; decreased bw gain days 6-9, no embryo- or foetal toxic effects, increased incidence of minor skeletal variants	the report states that the incidences of skeletal variants was significantly higher in styrene litters than controls; but within historical ranges; no data presented
Murray *et al.* (1978)	New Zealand White rabbit (n=20)	inh	gestation days 6-18, sacrifice day 29	0, 300 and 600 ppm 7h/day (0, 1299 and 2598 mg/m^3)	*300 ppm*: no maternal, embryo or foetal toxic effects *600 ppm*: no maternal toxicity, embryo or foetal toxic effects, increased incidence of minor skeletal variants	The report states that the incidence of minor skeletal variants was within the range normally observed among control groups from other recent studies

bw= body weight h= hour inh= inhalation gav= gavage bw= body weight no.= number

Table 2.2 Developmental toxicity studies with styrene.

authors	species	route	experimental pe-riod	dose	findings	remarks
Murray *et al.* (1978)	Sprague Dawley rat (n=29-39)	gav	gestation days 6-15, sacrifice day 21	0, 180 and 300 mg/kg bw (0, 90 and 150 mg/kg bw twice daily)	*180 mg/kg* maternal toxicity; decreased bw gain days 6-9, no embryo- or foetal toxic effects *300 mg/kg*: maternal toxicity; decreased bw gain days 6-9, no embryo- or foetal toxic effects.	the report states that the incidences of skeletal variants was significantly higher in styrene litters than controls; but within historical ranges, no data presented
Beliles *et al.* (1985)	COBS (SD)BR rats (control: 76 male and 106 females styrene: 50 male and 70 females)	drink	premating, mating, gestation and lactation during 3 generations	0, 125 and 250 ppm (~14 and 21 mg/kg bw)	*125 ppm*: no effects *250 ppm*: F1-pups: day 21 survival index decreased*, F2-pups: reduced pup survival days 1, 7, and 14, F2-females: reduced number of females which produced litters**, F3-pups: no effects on survival	*all dead pup were in two litters **2 of 5 females that failed to litter were mated to the same male, when corrected for this male the values were comparable with the controls
Zaidi *et al.* (1985)	Albino rats (n=6)	gav	1)throughout pregnancy and 2) throughout pregnancy and lactation 3) lactation alone	0 and 200 mg/kg bw	*200 mg/kg bw* 1)pregnancy: no effects 2)pregnancy+lactation: increases in norephydrine and serotinine levels and in striatal dopamine receptors 3)lactation: increases in norephydrine and serotinine levels and in striatal dopamine receptors	only 3 litters/group

bw = body weight h= hour inh= inhalation gav=gavage bw= body weight n= number of animals per group

Table 2.3 Developmental toxicity studies with styrene.

authors	species	route	experimental period	dose	findings	remarks
Srivastava *et al.* (1990)	Wistar rats (n=10)	gav	gestation days 6-15, sacrifice day 20	0, 250 and 400 mg/kg bw	*400 mg/kg bw*: decreased maternal body weight; decreased no, of implantations: increased no, of resorptions, decreased foetal weight. No skeletal malformations	maternal toxicity inadequate reported. Pre-implantation loss increased although exposure started at day 6 of gestation. Foetal viscera not examined. Foetal weight was determined in 50 selected foetuses/group
Daston *et al.* (1991)	Sprangue Dawley rats (n=16-18)	gav	GD 11 sacrifice GD 12 and 20	0,300 mg/kg bw	decreased food consumption and maternal body weights No developmental toxicity	preliminary results with a small number of litters
Kishi *et al.* (1995)	Wister rats (n=2- 5 litters)	inh.	GD 7-21 sacrifice ?	0, 217 and 1299 mg/m^3 for 6h/day	no maternal toxicity. Significant dose-dependent effects in tests performed prior to weaning (surface righting, pivoting locomotion and bar holding) as well as in tests performed after weaning (motor co-ordination, open-field behaviour and motor activity)	
Katakura *et al.* (1999)	Wistar rats (n=10-14 litters)	inh.	GD 6-20 sacrifice: PN day 0 and 21	0, 217 and 1299 mg/m^3 for 6h/day	maternal toxicity not described. Neurochemical effects in the brain of the developing pups	
Waalkens (1999b)	Wistar rats fermales (n=22 mated females/group)	gav	GD 6 to PN day 14. Sacrifice F1-pups PN days 4 and 21 Reproduction capacity of F1-males and females of the 200 mg/kg group. Sperm parameters F1-males of the 200 mg/kg group	0, 50, 100 or 200 mg/kg bw	*100 and 200 mg/kg bw group*: effect body weight during gestation. No other effect reproduction and litters. No effects on sexual maturation, oestrus cycle, reproduction capacity and sperm parameters	

bw = body weight h= hour inh= inhalation gav= gavage bw= body weight, n= number of animals per group; GD-gestation day; Pn-post natal day

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Calculation safe levels of styrene 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 styrene as an adult.

The RIVM (Jan94) proposed an ADI of 77 µg/kg body weight/day.
Maximal permissible level per infant is 346 µg/infant/day.
Maximal permissible level in breast milk is 385 µg/l 0.39 mg/l.

In conclusion, the committee considers 0.4 mg styrene/l breast milk as a maximal permissible level.

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