# Tetrachloroethylene (PER)

Evaluation of the effects on reproduction, recommendation for classification

**Gezondheidsraad** **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies 'Tetrachloroethylene (PER)'
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Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/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 en 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 tetrachloorethyleen (PER). Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Minister van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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# Tetrachloroethylene (PER)

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. 2003/04OSH, The Hague, February 18, 2003

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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# Contents

# Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie tetrachloorethyleen 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 tetrachloorethyleen de fertiliteit niet schaadt. Zij adviseert daarom om tetrachloorethyleen niet te classificeren.
- Voor effecten op de ontwikkeling adviseert de commissie tetrachloorethyleen in categorie 3 (s*toffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens*) te classificeren en met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om tetrachloorethyleen 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 tetrachloroethylene. The committee's recommendations are

- For effects on fertility, the committee recommends not to classify tetrachloroethylene 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 tetrachlororethylene in category 3 (*substances which cause concern for humans owing to possible developmental effects*) and to label tetrachloroethylene 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 tetrachloroethylene 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 tetrachloroethylene 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 Institute, 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 2002, 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 characterisation 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 leads to exceedence of the exposure limit for the general population, eg the acceptable daily intake (ADI).

## 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

* Organisation for Economic Cooperation and Development

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 summarised in Annex D.

## 1.6 Presentation of conclusions

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

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

## 1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises 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

# Tetrachloroethylene

## 2.1 Introduction

| | | |
|---|---|---|
| Name | : | Tetrachloroethylene |
| CAS-no | : | 127-18-4 |
| Synonyms | : | Tetrachloroethene, perchloroethylene, perchlor, PER, 1,1,2,2-tetrachloroethylene |
| Use | : | organic solvent, dry cleaning, degreasing |
| Mol weight | : | 165.8 |
| Chem formula | : | $C_2Cl_4$ |
| Conversion factor | : | 1 ppm = 6.89 mg/m$^3$ (101 kPa, 25ºC) |

## 2.2 Human studies

### Fertility

Taskinen *et al*. (Tas89) conducted a nested case-control study with 120 cases of spontaneous abortion and 251 controls. This study was based on 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 covering the

period of spermatogenesis. The incidence of spontaneous abortions among wives of men occupationally exposed to organic solvents was statistically significantly increased (cases 103, referents 182; crude odds ratio 2.7 (95% CI 1.3-5.6)). No effects on the incidence of spontaneous abortions among wives of men occupationally exposed to tetrachloroethylene (cases n=4, referents=17; crude odds ratio 0.5 (95% CI 0.2-1.5)) or halogenated hydrocarbons was observed.

Zielhuis and Van Der Gulden (Zie89) investigated the effect of tetrachloroethylene on the menstrual cycle in a small exploratory study. A self-administered questionnaire was used to obtain information about the menstrual disorders. The questionnaires of 68 'exposed' dry-cleaning workers and 76 'reference' laundry workers were used for analysis. There was no difference between the groups in mean cycle length. However, the incidences of dysmenorrhoea, unusual cycle length, menorrhagia and premenstrual syndrome were higher among dry-cleaning workers than among laundry workers. To the committee's opinion the significance of these findings is limited due to small sample size and the lack of exposure data.

Eskenazi *et al*. (Esk91a) studied the semen quality of 34 men exposed to tetrachloroethylene (dry-cleaning workers) and 48 laundry workers who were not exposed to tetrachloroethylene. As an index of exposure expired air levels of tetrachloroethylene were measured. Although no difference was observed between the two groups in the average number of sperm cells (> 80 million cells/ml), the incidence of men who were oligospermic (< 20 million cells/ml) was relative high (ca. 25%) in both groups. Furthermore, only subtle differences in sperm quality were observed between the groups.

In a second study of Eskenazi *et al*. (Eks91b), the pregnancy outcomes of the wives of the dry-cleaning workers and of the laundry workers who participated in the study described above (Eks91a) were examined. In total 17 partners of dry-cleaning workers and 32 partners of laundry workers participated in this study. The number of pregnancies and the standardized fertility ratios were similar between the two groups. Moreover, there was no difference in the incidence of spontaneous abortions between the groups. However, wives of dry-cleaning workers were more than twice as likely to have a history of attempting to become pregnant for more than 12 months or to have sought care for an infertility problem. This difference was not statistically significant. Furthermore, data about the correlation between length of time to conception and tetrachloroethylene exposure were unreliable and no association could be established between the length of time to conception and semen quality.

In a retrospective study, time to pregnancy was studied 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 (Sal95). 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). Daily or high solvent exposure, adjusted for potential confounding factors, was significantly associated with reduced fecundity (incidence density ratio [IDR] of clinical pregnancies was 0.41 (CI 0.27-0.62)). The IDR's for workers exposed to tetrachloroethylene were 0.63 (CI 0.34-1.17) (low exposure, n=13) and 0.69 (CI 0.31-1.52) (high exposure, n=7).

### Development

Hemminki *et al*. (Hem80) compared the incidence of spontaneous abortions among Finnish chemical workers in 1973-1976 with that of the general population. The information about the workers was obtained from files of the Union of Chemical Workers (n=9000) and the information about abortions was obtained from the Hospital Discharge Registry of the National Board of Health. The incidence of spontaneous abortions among laundry workers was statistically significantly increased when compared to the incidence of spontaneous abortions among all women in Finland. Data about exposure levels were not presented.

Lindbohm *et al*. (Lin84) analysed the incidence of spontaneous abortions between 1973-1976 after occupational exposure (to solvents, automobile exhaust fumes, polycyclic aromatic hydrocarbons, metals, textile dust, animal microorganisms and other chemicals) of women and their husbands. Information about the occupations was obtained from the 1975 national populations and housing census and the information about abortions was obtained from the Finnish Hospital Discharge Registry of the National Board of Health. No effect on the incidence of spontaneous abortions was observed among solvent exposed women (n=730 pregnancies; adjusted odds ratio 0.79 (95% CI 0.58-1.07)) and among the wives of solvent exposed husbands (n=1316 pregnancies; adjusted odds ratio 0.86 (95% CI 0.69-1.08)). The incidence of spontaneous abortions among female laundry workers was statistically significantly increased (n=416 pregnancies; adjusted odds ratio 1.48 (95% CI 1.09-2.02)). Data about exposure levels were not presented.

Bosco *et al*. (Bos87) interviewed 67 female workers in 53 dry-cleaning shops in Rome, Italy. The women reported 102 pregnancies of which 56 occurred during periods of their life of employment in dry-cleaning shops and 46 during periods they were housewives. Although the incidence of spontaneous abortions among women working in dry-cleaning shops was 4 times higher than among housewives, no statistically significant differences were observed in the incidences of low birth weights, spontaneous abortions, still births and congenital birth defects between the two groups. Mean trichloroacetic acid (an urinary metabolite of tetrachloroethylene but also of

trichloroethylene) levels in the urine among dry cleaners were four times higher than among women doing only ironing or among controls. However, in all groups the metabolite levels were low, suggesting rather low exposure levels.

McDonald *et al*. (Don87) performed a large cross-sectional study among 56067 Canadian women who delivered or were treated for a spontaneous abortion in 11 Montreal hospitals between1982-1984. All women were interviewed in detail regarding their occupational, social and personal characteristics in their most recent and past pregnancies (104649 pregnancies in total). The data were analysed in relation to 4 main adverse pregnancy outcomes; spontaneous abortion, stillbirth, congenital defects and low birth weight. The expected numbers of these 4 adverse pregnancy outcomes were calculated and compared with the observed numbers. In this cohort, 202 pregnancies occurred in women working in laundries or dry-cleaning shops. For none of the above mentioned adverse pregnancy outcomes, the observed to expected ratio was statistically significantly increased.

Kyyrönen *et al*. (Kyy89) defined a cohort of 5700 female dry-cleaning and laundry workers from the registers of the Union of Chemical Workers and of the Municipal Workers Union of Finland for the period of 1973-1983. Linking this file of study subjects with the files of the Hospital Discharge Register and the Finnish Register of Congenital Malformations resulted in a final study population of 130 cases of spontaneous abortion (and 289 controls) and 24 cases of malformations (and 93 controls). Statistical analysis showed a statistically significant association between the incidence of spontaneous abortion and high exposure (work tasks included dry cleaning for at least one hour daily on average or when the women reported handling of tetrachloroethylene at least once a week) to tetrachloroethylene (9 cases, 6 controls; adjusted odds ratio 3.4 (95% CI 1.0-11.2)). No effect of tetrachloroethylene exposure on the incidence of congenital malformations was observed.

In the study of Taskinen *et al*. (Tas89, for a description see section fertility), no association was observed between paternal exposure to solvents and the incidence of congenital malformations. Furthermore, no significant effect on the incidence of spontaneous abortions was observed after maternal exposure to organic solvents (cases n=11, referents n=18; adjusted OR 1.2 (95% CI 0.5-2.7)).

Ahlborg (Ahl90) investigated the risk of adverse pregnancy outcome (spontaneous abortion, perinatal death, congenital malformations and low birth weight) in two cohorts of women engaged in laundry or dry-cleaning work. A primary study consisted of 48 cases and 110 referents and a complementary study consisted of 68 cases and 131 referents (these women were identified in the Swedish Medical Birth Registry and in the Swedish Registry of Congenital Malformations). Statistical analysis of the total material did not show any effect after low (adjusted odds ratio 1.1 (95% CI 0.6-2.2)) or high

(adjusted odds ratio 1.1 (95% CI 0.5-2.2)) tetrachloroethylene exposure during the first trimester on the incidence of adverse pregnancy outcomes.

Lindbohm *et al*. (Lin90) identified a cohort of women who were biologically monitored for exposure to organic solvents between 1965-1983. Pregnancies occurring in this group between 1973-1983 were identified from the Finnish Register of Congenital Malformations. The final study material included 73 cases of spontaneous abortion and 167 controls. The incidence of spontaneous abortions in the group of women exposed to organic solvents (57%) during the first trimester of pregnancy was increased when compared to the control group (42%, odds ratio 2.2 (95% CI 1.2-4.1)). The incidence of spontaneous abortions in cases and controls exposed to tetrachloroethylene was 11% and 9%, respectively (odds ratio 1.4 (95% CI 0.4-4.2)). The odds ratios for the low- and high-exposure groups were 0.5 (95% CI 0.1-2.9) and 2.5 (95% CI 0.6-10.5), respectively. The odds ratio for spontaneous abortion for dry-cleaning workers exposed to tetrachloroethylene was 2.7 (95% CI 0.7-11.2), for other work in dry cleaning plants the odds ratio was 0.6 (95% CI 0.1-5.5) and for other work were women are exposed to tetrachloroethylene the odds ratio was 1.3 (95% CI 0.3-6.6).

Windham *et al*. (Win91) performed a case control study in California using women who had a spontaneous abortion at less than 20 weeks gestation each matched to two controls who had have a live birth. A significant association was observed between the incidence of spontaneous abortions and exposure to tetrachloroethylene (crude odds ratio 4.7 (95% CI 1.1-21.1)). However, these results were based on only 5 cases and 2 controls, and 4 of these women were also exposed to trichloroethylene.

Doyle *et al*. (Doy97) performed a retrospective occupational study of reproductive outcome in 7305 women who were currently or previously employed in dry cleaning or laundry units in the United Kingdom. Data about exposure concentrations to tetrachloroethylene were not presented. The rate of spontaneous abortion varied according to the type of work the women did during the pregnancy or in the three months before conception: being lowest for pregnancies not exposed to either dry cleaning or laundry work (10.9%), higher for those exposed to laundry work (13.4%) and higher still for those exposed to dry cleaning work (14.8%). Within the group of pregnancies exposed to dry cleaning, the proportion was higher if the women reported that she works as an operator at the time of pregnancy (17.1%) rather than as a non-operator (11.6%). Adjusted odds ratios for the period 1980-1995 showed that the risk was over 50% higher in operators than non-operators (P=0.04).

### Lactation

Bagnell *et al*. (Bag77) reported a case of a 6 weeks old breast-fed infant suffering from obstructive jaundice and hepatomegaly. The mother was exposed to tetrachloroethylene

as a result of lunching with her husband at the dry cleaning plant where he was employed. One hour after her lunch visit the breast milk of the mother contained 10 mg tetrachloroethylene/l. Avoidance of exposure to solvent vapours for the next 24 hours allowed the breast milk concentration to decrease to 3 mg/l. Cessation of breastfeeding resulted in rapid improvement of the infant's condition. Data about exposure levels were not presented.

Schreiber (Sch92, Sch93) modelled the infant exposure to tetrachloroethylene by breast milk feeding based on a variety of maternal occupational and residential inhalation exposure scenarios. The predicted concentration of tetrachloroethylene in breast milk of women ranged from 857-8440 µg/l for women occupationally exposed to tetrachloroethylene. For women exposed to tetrachloroethylene in residences near dry cleaners, the predicted breast milk concentrations of tetrachloroethylene ranged from 16-3000 µg/l and exposure to indoor residential background concentrations of tetrachloroethylene resulted in a predicted breast milk concentration of 1.5 µg tetrachloroethylene/l. These breast milk concentrations resulted in infant exposure levels of 0.0001 mg/kg body weight/day (1.5 µg/l milk) or 0.82 mg/kg body weight/day (8440 µg/l milk) assumed that a 7.2 kg infant ingests 700 ml milk a day.

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 tetrachloroethylene of 25 ppm (= 172 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 tetrachloroethylene ingested by a nursing infant over a 24-hour period was 1.36 mg in 0.92 l (1.48 mg/l).

## 2.3 Animal studies

Tables 1 and 2 (annex D) summarize the fertility and developmental studies with tetrachloroethylene in experimental animals.

### Fertility studies

Beliles *et al*. (Bel80) exposed rats and mice to 0, 100 or 500 ppm of tetrachloroethylene (0, 689, 3445 mg/m$^3$) for 5 consecutive days, 7 hours/day. An increased proportion of sperm with aberrant morphology was found in mice (but not in rats) after exposure to

500 ppm 4 weeks after exposure (19.7% in tetrachloroethylene group and 6% in control group). In mice, 10 weeks after exposure, there were no statistically significant differences in the proportion of abnormal sperm between the control group and the groups treated with tetrachloroethylene. In a dominant lethal assay performed on the rats, no adverse effects were observed.

Tinston (Tin95) performed a two-generation study, based on a standard protocol. Alpk:APfSD rats were exposed by inhalation to 0, 100, 300 or 1000 ppm tetrachloroethylene (0, 689, 2067, 6890 mg/$m^3$) 5 days/week, 6 hours/day for 11 weeks prior to mating. During and following mating, F0-males were exposed daily (6 hours/ day) until termination and F0-females were exposed until gestation day (GD) 20. One F1A litter was produced; dams together with their litter were exposed daily from postnatal day (PN) 6 to 29. The second generation parents (F1) were selected from the F1A litters on PN 29 and were exposed to tetrachloroethylene for at least 11 weeks prior to mating. Two litters F2A and F2B were produced in the second generation. F2A litters were exposed from PN 6-29 (0 and 100 ppm) or PN 7 to 29 (300 ppm). Dams and litters of the 1000 ppm group were not exposed. F2B litters were generated by mating males and females of the control, 300 and 1000 ppm groups. There was no exposure of the dams and F2B litters during lactation. An F2C generation was obtained after mating F1 males of the control and 1000 ppm to non exposed females. Although in the 1000 ppm group of the F0 generation, some statistically significant reductions in body weight were observed during the premating, gestation and lactation periods, these effects were only marginal. In the F1-generation a more pronounced effect on body weight was observed in this group mainly due to the low pup weight on PN 29 (initial weight at the start of the premating period). During the first 2 weeks of exposure to 1000 ppm in each generation (F0 parents and F1A pups) a decreased activity and reduced response to sound was observed. These signs were not present approximately 30 minutes after the end of exposure. In a few animals of the 1000 ppm group clinical observations such as salivation, breathing irregularities, piloerection and tip-toe gait were observed. Kidney and liver weight of the males of the 1000 ppm group were significantly increased. In the 1000 ppm group, histological changes (slight nuclear pleomorphism) were observed in the kidneys of the males in both generations and in the females of the F0-generation. A statistically significant dose-related reduction in the testis weight was observed in the 300 and 1000 ppm group in the second generation, but there were no associated histopathological changes in the 1000 ppm group. There were no effects on fertility in any of the exposed groups from either generation.

## Developmental toxicity

Tinston (1995) performed a two-generation study, based on a standard protocol. Alpk:APfSD rats were exposed by inhalation to 0, 100, 300 or 1000 ppm tetrachloroethylene (0, 689, 2067, 6890 mg/$m^3$) 5 days/week, 6 hours/day for 11 weeks prior to mating. During and following mating, F0-males were exposed daily (6 hours/ day) until termination and the F0-females were exposed until gestation day (GD) 20. One F1A litter was produced; dams together with their litter were exposed daily from postnatal days (PN) 6 to 29. The second generation parents (F1) were selected from the F1A litters on PN 29 and were exposed to tetrachloroethylene for at least 11 weeks prior to mating. Two litters F2A and F2B were produced in the second generation. F2A litters were exposed from PN 6-29 (0 and 100 ppm) or PN 7 to 29 (300 ppm). Dams and litters of the 1000 ppm group were not exposed. F2B litters were generated by mating males and females of the control, 300 and 1000 ppm groups. There was no exposure of the dams and F2B litters during lactation. An F2C generation was obtained after mating F1 males of the control and 1000 ppm to non exposed females. Although in the 1000 ppm group of the F0 generation some statistically significant reductions in body weight were observed during the premating, gestation and lactation periods, these effects were only marginal. In the F1-generation a more pronounced effect on body weight was observed in this group mainly due to the low pup weight on PN 29 (initial weight at the start of the premating period). During the first 2 weeks of exposure to 1000 ppm in each generation (F0 parents and F1A pups) a decreased activity and reduced response to sound was observed. These signs were not present approximately 30 minutes after the end of exposure. In a few animals of the 1000 ppm group clinical observations such as salivation, breathing irregularities, piloerection and tip-toe gait were observed. In the 1000 ppm group of the first generation a slight effect was observed on the number of pups born alive and pup survival (PN 5-22). Pups of the 1000 ppm group showed signs of sedation after exposure on PN 6 up to PN 29. Pup weights of the 300 and 1000 ppm were reduced when compared to the controls. In the second generation in the F2A and F2B litters of the 1000 ppm group a statistically significant effect was observed on the proportion of pups born alive and pup survival (PN 1-5 and 5-22). The total number of pups born (live and dead pups) per litter in the 1000 ppm group in the F2A litters and more pronounced in the F2B litters was decreased; the authors did not calculate this parameter nor performed statistics (F2A: control group 11.9 versus 1000 ppm group 10.1; F2B control group 10.6 versus 1000 ppm group 7.9). Pup weights of F2A and F2B litters were decreased in the 1000 ppm group. No significant effects were observed in the F2C litters; this indicates that the effects observed in the 1000 ppm group were not male mediated.

Schwetz *et al*. (Sch75) studied the developmental effects after inhalation of tetrachloroethylene (300 ppm=2067 mg/m$^3$) during gestational days 6-15, for 7 hours/ day in Sprague Dawley rats and Swiss Webster mice. Increased maternal relative liver weights of mice and slight but statistically significant decreases in rat body weights were observed. In mice, a decrease in fetal body weight, retarded ossification of the skull bones and sternebrae was observed. In rats, the resorption rate in the tetrachloroethylene group was increased to 9% versus 4% in the controls. These effects might be caused by maternal toxicity or by chance, since, for some parameters, a high variance between 2 described control groups was observed.

Organic materials concentrated from the drinking waters of five US cities selected as representative of the major sources of raw water were administered to groups of pregnant CD-1 mice from GD 7-14 by gavage at dose levels representative for 300, 1000 and 3000 times the anticipated human exposure to these materials (Kav79). The tetrachloroethylene exposure level in the highest dose group was calculated to be 0.007 mg/kg body weight /day. In the drinking-water concentrates also other compounds, e.g. chloroform [calculated exposure level 7.1 mg/kg body weight], were present. The dams were killed on GD 18 and the fetuses were examined for skeletal and visceral anomalies (Kav79). Except from slight effects on body weights and relative liver weight, no maternally toxic effects were observed. Furthermore, no effects were observed on the fetuses.

Beliles *et al*. (Bel80, see also the section about fertility) found no maternal toxicity or developmental effects after exposure by inhalation to 500 ppm (= 3445 mg/m$^3$) tetrachloroethylene, 7 hours /day, at gestational days 0-18 or 6-18, with or without a 3-week pregestational exposure period in Sprague Dawley rats, except for a increased maternal kidney weight in the group treated premating and during gestational days 6-18.

The same investigators (Bel80) also exposed female New Zealand White rabbits by inhalation to 500 ppm tetrachloroethylene (7 hours/day, gestational days 0-21 or 7-21, with or without a 3-week pregestational exposure). No maternal toxicity, fetal toxicity or teratogenic effects were reported.

In a behavioural study, Nelson *et al*. (Nel80) exposed pregnant Sprague-Dawley rats to 0, 100 and 900 ppm (0, 689, 6201 mg/m$^3$) of tetrachloroethylene for 7 hours/day on gestational days 7-13 or 14-20. Various behavioural tests were performed on the offspring on postnatal days 4-56 and histological and biochemical examinations of the pups brain was performed. Exposure to 900 ppm (both periods) caused decreased maternal weight gain, reduced food consumption. Inconsistent results were obtained in the behavioural tests and biochemical analysis of the brain of 21 day old pups demonstrated significant reductions in the levels of acetylcholine for the group whose dams were exposed during days 7-13 of gestation.

Smith *et al*. (Smi89) exposed Long Evans rats to 0, 330, 800, 1200 or 1800 mg/kg trichloroacetic acid (a metabolite of tetrachloroethylene) by gavage on days 6-15 of gestation. Maternal weight gain was reduced at 800, 1200 and 1800 mg/kg and a dose-related increase in spleen and kidney weights was observed which was statistically significant at all dose levels. Embryolethality was significantly increased in the three highest dose groups. Foetal weight and foetal length were statistically significantly dose-related decreased in all treatment groups. The incidence of soft tissue malformations, mainly in the cardiovascular system, was dose-related and statistically significantly increased in all dose groups. The incidence of skeletal malformations was statistically significant increased in the 1200 and 1800 mg/kg groups.

### Lactation

No animal studies on the effects on lactation were found.

## 2.4 Conclusion

Occupational exposure to mixtures of organic solvents has been shown to increase the incidence of spontaneous abortions among wives of exposed men (Tas89) and to decrease fecundity of exposed women (Sal95). However, in the study of Taskinen *et al*. (1989) no effect of tetrachloroethylene exposure on spontaneous abortions was observed. In the study of Sallmén *et al*. (Sal95) an effect of tetrachloroethylene exposure on fecundity was observed but the groups were very small (n=7 or 13) and no dose response relationship was observed. Zielhuis and Van der Gulden (Zie89) observed an increased incidence of menstrual disorders among dry-cleaning workers but the significance of these findings was limited due to the small sample size and lack of exposure data. In the studies of Eskenazi *et al*. (Esk91a, Esk91b) subtle effects on sperm parameters were observed in dry-cleaning workers (Esk91a) but there were no effects on fertility (Esk91b). Although effects were observed on fertility in men, it is not clear whether the described effects are due to tetrachloroethylene exposure alone, or to a mixture of compounds. For this reason, the committee recommends not to classify tetrachloroethylene with respect to effects on fertility because of a lack of appropriate human data.

In the animal studies, Beliles *et al*. (Bel80) found an increased incidence of aberrant sperm morphology in mice (but not rats), 4 weeks after tetrachloroethylene exposure. However, after 10 weeks no effect was observed. In addition, in the two-generation study of Tinston *et al*. (Tin95), no effect of tetrachloroethylene on fertility was observed (but maternal toxicity was observed).

In conclusion, based on the data from animal studies, the committee is of the opinion that sufficient data show that no classification for effects of tetrachloroethylene on fertility is indicated.

Hemminki *et al*. (Hem80) and Lindbohm *et al*. (Lin84) reported an increased incidence of spontaneous abortions among laundry workers. However, no data about tetrachloroethylene-exposure levels in the laundries were presented in these studies (tetrachloroethylene is particularly used by dry-cleaning workers and to a lesser extent in laundries). Bosco *et al*. (Bos87), Mc Donald *et al*. (Don87), Taskinen *et al*. (Tas89), Ahlborg *et al*. (Ahl90), Lindbohm *et al*. (Lin90) observed no statistically significant adverse effects on pregnancy outcome. In the study of Kyyrönen *et al*. (Kyy89) and of Windham *et al*. (Win91) a significant association was observed between (high) exposure to tetrachloroethylene and the incidence of spontaneous abortions. However, the results of both studies were based on relatively small groups and in the study of Windham et al. (Win91) women reported that they were also exposed to trichloroethylene. Doyle *et al*. (Doy97) showed that women working in dry-cleaning shops, and especially those who were working as an operator, had an increased risk for having a spontaneous abortion. However, since exposure levels of tetrachloroethylene were not measured and the data were not corrected for confounding factors such as heavy lifting this study showed only an association between job and incidence of spontaneous abortions.

In conclusion, the committee is of the opinion that although effects were observed on development in man, it is not clear whether the described effects are due to tetrachloroethylene exposure alone, or to a mixture of compounds.

In animal studies, Schwetz *et al*. (Sch75) observed effects of inhalatory tetrachloroethylene exposure (300 ppm) on ossification in mice and on the incidence of resorption in rats. However, these effects were observed at dose levels which induced maternal toxic effects as well. In the study of Beliles *et al*. (Bel80), no significant developmental effects after inhalatory tetrachloroethylene exposure (500 ppm) were observed in rats and rabbits. In a two-generation study of Tinston *et al*. (Tin95), tetrachloroethylene had an effect on the number of F1- and F2-pups born alive, pup survival and pup body weights. Furthermore, the total number of F2-pups born (live and dead pups) per litter was decreased. In this study general toxicity (effect on body weight (F0 and F1) and histopathological effects on kidneys (only F0)) was observed in the F0- and F1-male parents and F0-females. Nelson *et al*. (Nel80) observed inconsistent effects on behavioural parameters and on biochemical parameters of the brain in the offspring of rats exposed to tetrachloroethylene at dose levels inducing maternal toxic effects.

In conclusion, in view of the animal studies, the committee recommends to classify tetrachloroethylene in category 3 ('substances which cause concern for humans owing to possible developmental toxic effects') and to label with R63 (may cause harm to the unborn child).

From the study of Fisher *et al*. (a pharmacokinetic lactation model), a concentration of 1.48 mg tetrachloroethylene/l breast milk was predicted (Fis97). The committee is of the opinion that this (predicted) tetrachloroethylene concentration in human breast milk can only be used as an indication for the possible concentration 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. In studies of Schreiber *et al*. (Sch92, Sch93), a maximal level of tetrachloroethylene in breast milk was predicted ( in a model) to be 8.44 mg/l. In addition, in the study of Bagnell *et al*. (Bag77) 10 mg tetrachloroethylene per litre breast milk was related to clinical signs observed in a 6-weeks old breast-fed infant.

No additional experimental data are available about the concentration of tetrachloroethylene in human breast milk and about the possible effects during lactation. Therefore the committee concluded that a lack of appropriate data precludes assessment of tetrachloroethylene for labelling for effects during lactation.

### Proposed classification for fertility

A lack of appropriate human data preclude the assessment of tetrachloroethylene and sufficient animal data show that no classification for tetrachloroethylene is indicated for effects on fertility.

### Proposed classification for developmental toxicity

Category 3, R63.

### Proposed labelling for effect during lactation

Lack of appropriate data precludes assessment of tetrachloroethylene for labelling for effects during lactation.

## Additional consideration

The committee would like to emphasise that several human studies considered here in view of tetrachloroethylene 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 tetrachloroethylene or a mixture of solvents containing tetrachloroethylene. Therefore, the EU Classification and Labelling guideline does not warrant a classification of tetrachloroethylene on the basis of these human studies. However, the committee emphasises that there is clearly cause for concern for effects on fertility and development after exposure to mixtures of solvents containing tetrachloroethylene.

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A The committee

B Comments on the public draft

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

D Fertility and developmental toxicity studies

E Abbreviations

# Annexes

Annex **A**

# De committee

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Annex **B**

# Comments on the public draft

A draft of the present report was released in 2002 for public review. The following persons and organisations have commented on the draft review:

- V Digernes, Federation of Norwegian Process Industries, Norway

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 results 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, peripostnatal defects, and impaired postnatalmental 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 exposue 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 in- dicate 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

*Table 1.1* Fertility studies with tetrachloroethylene in experimental animals.

| authors | species | experimental period/ design | dose and route | general toxicity | effects on reproductive organs/effects on reproduction | remark |
|---|---|---|---|---|---|---|
| Beliles *et al*. (1980) | SpragueDawley rats(n=?) | 7 h/d for 5 consecutive days | 0, 689 and 3445 mg/m$^3$ by inh | not presented | No effect on sperm head morphology | |
| Beliles *et al*. (1980) | CD-1 mice(n=?) | 7 h/d for 5 consecutive days | 0, 689 and 3445 mg/m$^3$ by inh | not presented | Increased anomalies in sperm head morghology after 4 w. in 3445 mg/m$^3$ group. | |
| Tinston (1995) | A1p1: APfSD rats (male and females 24/sex/ group) | 11 weeks prior to mating, mating, GD 0-20 andPN 6-29 (see text for exposure during lactation) Males of F0 and F1 generation exposed for 19 and 35 w, respectively before sacrifice | 0, 689, 2067 or 6890 mg/m$^3$ 6h/day; prior to mating 5d/ week and daily during mating, gestation and lactation by inh | 6890 mg/m$^3$ group:decreased BW in F0- and F1-generation; increased kidney and liver weights; histological changes in kidneys in males of both generations and in females of the F0-generation | 2067 mg/m$^3$ : reduced testis weight F1-males; no effect on fertility 6890 mg/m$^3$ : reduced testis weight F1-males; no histopathological changes; no effect on fertility | two-generation study |

bw = body weight h=hour d= day w=week inh=inhalation gav=gavage n=number GD=gestation day

*Table 2.1* Developmental toxicity studies with tetrachloroethylene in experimental animals.

| authors | species | experimental period/design | dose and route | general toxicity | developmental toxicity | remarks |
|---|---|---|---|---|---|---|
| Schwetz *et al*.(1975) | Swiss Webster-mice(n=17) | GD 6-15; sacrifice day 18 | 0 and 2067 mg/$m^3$ 7 h/day by inh | relative liver weight increased | decreased fetal weight, delayed ossification skull bones and sternebrae | |
| Schwetz *et al*.(1975) | Sprague Dawley rats (n=17) | GD 6-15; sacrifice day 21 | 0 and 2067 mg/$m^3$, 7h/day by inh. | decreased body weight | increased no. resorptions | |
| Kavlock *et al*.(1979) | CD-1 mice (number of litters: 11-72) | GD 7-14; sacrifice day 18 | by gav, see remark | | no effects | 300, 1000 and 3000 x the human exposure to the levels in drinking water; the highest dose corresponded to 0.007 mg/kg bw |
| Beliles *et al*.(1980) | Sprague Dawley rats (n=19-24) | GD 0-18 or 6-18 with or without 3-wk premating; sacrifice day 21 | 0 and 3445 mg/$m^3$ 7h/day by inh. | no maternal toxicity except for increased kidney weight in group GD 6-18 with 3-wk premating exposure | 3445 mg/$m^3$: no fetal toxicity and teratogenicity | |
| Beliles *et al*.(1980) | New Zealand White rabbits | GD 0-21 or 7-21 with or without 3-wk premating; sacrifice day 30 | 0 and 3445 mg/$m^3$ 7h/day by inh | no maternal toxicity | 3445 mg/$m^3$ : no effects fetal toxicity and teratogenicity | |

bw = body weight h=hour d= day w=week inh=inhalation gav=gavage n=number GD=gestation day

*Table 2.2* Developmental toxicity studies tetrachloroethylene in experimental animals.

| authors | species | experimental period/design | dose and route | general toxicity | effects on reproductive organs/effects on reproduction | remarks |
|---|---|---|---|---|---|---|
| Nelson *et al*(1980) | Sprague Dawley rats (n=19) | GD 7-13 or 14-20 | 0, 689 and 6201 mg/m$^3$ 7h/day by inh. | decreased maternal weight gain, reduced food consumption | GD 7-13, 6201 mg/m$^3$ decreased performance in some behavioural tests; reduced levels of acetylcholine in the brain 21-day old pups GD 14-20, 6201 mg/m$^3$ decreased performance of pups in some behavioral tests; in other test superior performance | None of the differences observed were consistent in time or impressive |
| Tinston(1995) | A1p1; APfSD rats (male and females 24/sex/group) | 11 weeks prior to mating, mating, GD 0-20 and PN 6-29 (see text for exposure during lactation) Males of F0 and F1 generation exposed for 19 and 35 w, respectively before sacrifice | 0, 689, 2067 or 6890 mg/m$^3$ 6h/day; prior to mating 5d/week and daily during mating, gestation and lactation by inh. | 6890 mg/m$^3$ group: decreased BW in F0- and F1-generation; increased kidney and liver weights; histological changes in kidneys in males of both generations and in females of the F0-generation | 6890 mg/m$^3$: decreased number of pups born and decreased pup survival 2067 and 6089 mg/m$^3$: reduced pup weights | |

bw = body weight h=hour d= day w=week inh=inhalation gav=gavage n=number GD=gestation day

Annex **E**

# Abbreviations

Abbreviations used:

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