Trichloroacetic acid

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies '*Trichloroacetic acid*'
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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 trichloorazijnzuur. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb deze publicaties heden ter kennisname aan de minister van Volksgezondheid, Welzijn en Sport, de minister van Sociale Zaken en Werkgelegenheid en de staatssecretaris van Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

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Trichloroacetic acid

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. 2006/03OSH, The Hague, June 13, 2006

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad trichloorazijnzuur (TCA) onder de loep genomen. TCA wordt toegepast bij de productie van zijn natriumzout, een onkruidverdelger. Daarnaast wordt TCA gebruikt als oplosmiddel in de plasticindustrie en als etsend middel in de metaalbewerking. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de raad, kijkt naar effecten op de vruchtbaarheid van mannen en vrouwen zowel als op de ontwikkeling van het nageslacht. Bovendien worden effecten van blootstelling van de zuigeling via de moedermelk beoordeeld.

Op basis van Richtlijn 93/21/EEC van de Europese Unie doet de commissie een voorstel voor classificatie. Voor TCA komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte gegevens beschikbaar zijn. Zij adviseert daarom om trichloorazijnzuur niet te classificeren.

- voor effecten op de ontwikkeling adviseert de commissie trichloorazijnzuur in categorie 2 (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*) te classificeren en met T;R61 te kenmerken.
- voor effecten tijdens de lactatie adviseert de commissie om trichloorazijnzuur niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report the Health Council of the Netherlands reviewed trichloroacetic acid (TCA). The main use of TCA is in the production of its sodium salt, which is a known herbicide. It is also used as an etching agent in metal surface finishing and as a solvent in the plastics industry. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be occupationally exposed. The Committee for Compounds toxic to reproduction, a committee of the Health Council, evaluates the effects on male and female fertility and on the development of the progeny. Moreover, the committee considers the effects of a substance on the lactation and effects on the progeny via lactation.

According to the Directive 93/21/EEC of the European Union, the committee recommends a classification. The committee's recommendations for TCA are:

- for effects on fertility, the committee recommends not classifying trichloroacetic acid on the basis of a lack of sufficient data.
- for developmental toxicity, the committee recommends classifying trichloroacetic acid in category 2 (*substances which should be regarded as if they cause developmental toxicity for humans)* and labelling trichloroacetic acid with T;R61.
- For effects during lactation the committee is of the opinion that due to a lack of appropriate data trichloroacetic acid should not be labeled.

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 trichloroacetic acid 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 Drs. MM Tegelenbosch-Schouten at the Toxicology and Applied Pharmacology department of TNO Nutrition and Food Research, Zeist, The Netherlands, by contract with the Dutch Health Council. 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 abovementioned 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 2005, 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 at 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 the 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 exceeded the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

1.5 Data

Literature searches were conducted in the on-line databases Current Contents and Medline, starting from 1966 up to 2004 and by searches on internet. 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 as well as several websites regarding (publications on) toxicology and health. References are divided in literature cited and literature consulted, but not cited.

The committee chose to describe both the human and animal studies in the text. The animal data are described in more detail in Annex D as well. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

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 (Niesink *et al.*)[1] 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 characterisation, human exposure assessment and recommendations of other organisations.

Chapter 2

Trichloroacetic acid (TCA)

2.1 Introduction

Name	:	Trichloroacetic acid
CAS-no	:	76-03-9
Synonyms	:	Trichloracetic acid Trichloroethanoic acid Trichloromethanecarboxylic acid[a]
Use	:	The main use is in the production of its sodium salt, which is used as a herbicide; also used as a pickling or etching agent in metal surface finishing; a swelling agent and solvent in the plastics industry; auxiliary in textile finishing; decalcifier and fixative in microscopy; protein precipitating agent in laboratories; additive in mineral lubricating oils; polymerization catalyst; intermediate in the chemical synthesis of esters; medical agent in treating skin disorders, to remove genital warts and as an astringent and antiseptic[a]
Mol weight	:	163.39 (IARC)[2]
Chem formula	:	$C_2HCl_3O_2$ (IARC)[2]
Conversion factor	:	1 ppm = 6.67 mg/m^3; 1 mg/m^3 = 0.150 ppm at 25°C (calculated) [a]
General toxicity	:	Trichloroacetic acid administered to Sprague Dawley rats in drinking water at levels of 0, 50, 500 and 5000 ppm for a period of 90 days induced substantial toxicity to the target organs liver and kidney at a dose level of 5000 ppm (Mather *et al.*)[3]. Based on the water consumption the estimated doses of trichloroacetic acid are 0, 4.1, 36.5 and 355 mg/kg/day.

Exposure limit	:	The current (administrative) occupational exposure limit for trichloroacetic acid in the Netherlands is 1 mg/m^3, 0.150 ppm[b]
Kinetics	:	Trichloroacetic acid (TCA) is the major metabolite of trichloroethylene (TCE) and is besides trichloroethanol (TCOH), a major urinary metabolite in man. In workers, both metabolites are used as markers of exposure to TCE.

a Cheminfo: Canadian Centre for occupational Health and Safety
b Nationale MAC-lijst 2005

Human *in vivo* studies have identified the major urinary metabolites of trichloroethylene to be trichloroethanol (-glucuronide) (~50% of the administered dose) and trichloroacetic acid (~19% of the administered dose[4]). In this report, literature is cited in which exposure to trichloroacetic acid was investigated or in which trichloroacetic acid as metabolite was determined in urine or seminal fluid. Literature with respect to trichloroethylene-exposure, in which trichloroacetic acid was not measured as a metabolite, is not cited in this report. Adverse effects seen in these studies, however are possibly caused by the metabolite trichloroacetic acid. In 2003, the present committee recommended not to classify trichloroethylene for its effects on fertility. For the effects on the development the committee recommended classifying trichloroethylene in category 2.

2.2 Human studies

Fertility

Chia *et al.*[5] examined the association between exposure to trichloroethylene and effects on spermatogenesis among a group of 85 workers in an electronics factory. Semen analysis included volume, sperm density, sperm viability, motility and morphology. Personal monitoring of environmental trichloroethylene exposure was conducted for 12 workers and urine was analysed for trichloroacetic acid (0.8 - 136.4 mg/g creatinine). The urine samples were collected on the end of the day at which ‘time’ the semen given. Since there was no control group in this study, the results of the semen analysis were compared with WHO criteria. According to these criteria, 71.8% of the subjects had a normal semen volume. Sperm density of 88.2% of the subjects and sperm motility of 64.7% of the subjects was normal. Sperm morphology was normal in 30.6% of the subjects. There were no differences in volume, motility and morphology among the high-exposure and low-exposure groups. The committee is of the opinion that the results of this study are inconclusive because of the lack of relevant exposure data and a control group.

Forkert *et al.*[6] analysed human seminal fluid and urine samples from eight mechanics (26-40 years old) diagnosed with clinical infertility and exposed to trichloroethylene occupationally for at least two years. A control group (seminal fluid samples from five subjects that did not use trichloroethylene) was included. Urine samples from only two of the eight subjects contained trichloroacetic acid (concentration was 0,42 and 4,22 μmol/1) and/or trichloroethanol (concentration was 0,89 μmol/1 or less than 0,60 μmol/l). In seminal fluid samples from all eight subjects trichloroethylene and several metabolites (chloral and trichloroethanol) were detected. Trichloroacetic acid was only identified in only one subject. As trichloroacetic acid was only detected in one urine and semen sample and only eight subjects were included in the study population, the committee is of the opinion that this study is not relevant for evaluating the effect of trichloroacetic acid.

Developmental studies

Lindbohm *et al.*[7] studied the association between medically diagnosed spontaneous abortions and maternal occupational exposure to organic solvents. The final population for the analysis was restricted to 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 women exposed to organic solvents (58%) compared to controls (42%) (OR 2.2; 95% CI 1.2-4.1). No association between trichloroethylene exposure (measured as amount of trichloroacetic acid in urine or by self reported exposure to trichloroethylene) and the incidence of spontaneous abortions was observed (OR, adjusted for confounding factors, was 0.6 (95% CI 0.2-2.3).

Lactation

No human data on lactation are available for trichloroacetic acid.

2.3 Animal studies

In tables 1 and 2 (Annex D), fertility and developmental studies performed in animals are summarised.

Fertility

Inhalation

Forkert *et al.*[8] described four groups of six male CD-1 mice which were exposed to *trichloroethylene* (1000 ppm, 5370 mg/m^3) by inhalation for 6 hours/day, 5 days/week for 4 consecutive weeks. To estimate internal exposure, trichloroethylene metabolites ie. trichloroacetic acid and trichloroethanol were measured in urine. Levels of trichloroacetic acid increased in time. Enzymes involved in the trichloroethylene metabolism were found in higher levels in epididymal epithelium than in testicular Leydig cells. After four weeks of trichloroethylene exposure, damage to the epididymis was manifested as sloughing of epithelial cells. It was indicated that trichloroethylene is metabolised to trichloroacetic acid and trichloroethanol in the male reproductive tract, leading to adverse effects in the epididymis. The authors stated that the toxicity has a high probability of affecting spermatozoa that develop and mature within the epididymal environment. Fertility parameters, however, were not investigated in this study.

Gavage

Manson *et al.*[9] exposed female Long Evans rats by gavage for 5 days/week during 2 weeks premating and 1 week mating and 7 days/week during 3 weeks of gestation to *trichloroethylene* in corn oil at exposure levels of 0, 10, 100 and 1000 mg/kg body weight/day (n=23 rats per treatment group). Treated females were mated with untreated males. Trichloroethylene and metabolite levels were analysed by gas chromatography at different time points, i.e. in non mated females at the end of the mating period; in culled offspring at postpartum day 3 and in dams and offspring sacrificed at postnatal day 28 and 31. A significantly reduced body weight gain was seen in the highest dose group during premating and one animal of the highest dose group died during this period. Gas chromatographic analysis of tissues from females at the end of premating exposure indicated that trichloroethylene levels were uniformly high in fat, adrenals and ovaries across treatment groups and that relatively high levels of trichloroacetic acid were measured in uterine tissue. Female fertility, however, was not influenced.

Zenick *et al.*[10] exposed male Long Evans rats (n=10 males/group) to 0, 10, 100 and 1000 mg *trichloroethylene*/kg body weight/day by gavage for 6 weeks (5 days a week). Corn oil was used as vehicle. At the end of week 1 and 5 of the

exposure period and 4 weeks post-exposure, males were allowed to mate with ovariectomized hormonally primed females and copulatory behaviour and semen evaluations were conducted. In week 1 an impaired copulatory behaviour was observed among the animals of the highest dose group. In week 5 the body weight gain at the highest dose level was reduced, while copulatory performance returned back to normal. Trichloroacetic acid was present in extremely low concentrations in the control animals and was dose related higher in groups treated with 10, 100 and 1000 mg/kg body weight/day at the sixth week of exposure. Semen evaluations (sperm count, morphology and motility) did not show any spermatotoxic effect. Furthermore, no effect was observed on testosterone levels. These data suggest that trichloroethylene exerts minimal direct effects on the male reproductive system in spite of elevated concentrations of trichloroethylene and its metabolites.

Nelson and Zenick[11] evaluated whether the effect of *trichloroethylene* on male copulatory behaviour was caused by the narcotic properties of the compound. Following an oral dose of trichloroethylene (1000 mg/kg body weight/day), an increased ejaculation latency was seen. Since naltrexone, an opiate antagonist, blocked this effect, the authors stated that the alterations seen in sexual behaviour may be attributed to the narcotic properties of the compound.

Developmental studies

Gavage

Smith *et al.*[12] exposed female Long Evans rats to 0, 330, 800, 1200 or 1800 mg/kg body weight/day *trichloroacetic acid* by gavage on days 6-15 of gestation. Distilled water was used as vehicle. Maternal weight gain was reduced after exposure to 800, 1200 and 1800 mg/kg body weight/day. In addition, a dose-related (statistically significant) increase in spleen and kidney weights was observed at all dose levels. Embryolethality was significantly dose-related 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 body weight/day groups. Besides, the incidence of microphthalmia and anophthalmia was increased in the trichloroacetic acid dosed groups.

Fisher *et al.*[13] treated female Sprague Dawley rats with oral doses of *trichloroethylene* (500 mg/kg body weight/day), *trichloroacetic acid* (300 mg/kg body weight/day) or *dichloroacetic acid* (300 mg/kg body weight/day) once a day during pregnancy days 6 through 15 in order to determine the effectiveness of these materials to induce cardiac effects in the foetus. All-trans retinoic acid, 15 mg/kg body weight/day, was used as positive control. Water was used as vehicle for trichloroacetic acid and dichloroacetic acid and in a control group. Retinoic acid and trichloroethylene were dissolved in soybean oil. A soybean control group was included in the study. On gestation day 21 necropsy was performed. Following CO_2 euthanasia, each foetus was examined under a stereomicroscope for in situ cardiovascular malformations. The procedure included thorough examinations of the pulmonary and aortic trunks, atrial septum, pulmonary and aortic valves, endocardial crushions, both atriventricular valves and the ventricular septum. Mean maternal body weight gain was significantly decreased (3 to 8%) in the trichloroacetic acid and dichloroacetic acid treated females during pregnancy, as compared to the water control group. No maternal toxicity was seen in the control-, retinoic acid- , and trichloroethylene- treated females. Besides in the trichloroacetic acid-treated females a reduced uterine weight was seen. All treatments, except trichloroethylene, resulted in a statistically significant fetal body weight reduction when compared to corresponding controls. Treatment with high doses of trichloroethylene, trichloroacetic acid and dichloroacetic acid did not result in a statistically significant increase in the incidence of heart malformations, on a per foetus or a per litter basis, when compared to controls. The only statistically significant increase in the incidence of heart malformations, based on a per foetus or per litter basis, was found in the retinoic acid treatment group.

Manson *et al.*[9] exposed female Long Evans rats by gavage during 2 weeks premating, 1 week mating (5 days/week) and during 3 weeks of gestation (7 days/week) to *trichloroethylene* in corn oil at exposure levels of 0, 10, 100 and 1000 mg/kg body weight/day (n=23 rats per treatment group). Treated females were mated with untreated males. Trichloroethylene and metabolite levels were analysed by gas chromatography at different time points, i.e. in non mated females at the end of the mating period; in culled offspring at postpartum day 3 and in dams and offspring sacrificed at postnatal day 28 and 31. A significantly reduced maternal body weight gain was seen in the high dose group during pregnancy and two animals of this group died during this period. In addition, one high-dose female died on the day of delivery. Neonatal survival was significantly decreased in the high dose group up to day 14 of lactation. The majority of deaths occurred within 3 litters among the female pups at the time of birth. A

dose-related increase of trichloroacetic acid levels was seen in female neonatal tissues (blood, liver and milk contents of the stomach) measured at day 3 of lactation.

Drinking water

Johnson *et al.*[14] studied the effect of various trichloroethylene metabolites on foetal cardiac teratogenic effects in rats. Sprague Dawley rats were given drinking water containing these metabolites during pregnancy. *Trichloroacetic acid* was given as a dosage of 2730 ppm in drinking water, corresponding to 291 mg/kg body weight/day. No signs of general toxicity were observed. Caesarean section was performed at day 22 of gestation. Post-implantation loss was significantly increased. Moreover, a significant increase in cardiac defects was seen (on individual and litter basis).

Lactation

Fisher *et al.*[15] performed an inhalation study, in which lactating rats were exposed to 610 ppm (3222 mg/m^3) *trichloroethylene* for 2 weeks (4 hours/day, 5 days/week) from days 3 to 14 of lactation. Twenty hours after exposure on day 11 and immediately after exposure on day 14, milk was collected and trichloroethylene and trichloroacetic acid was analysed. The measured concentration of trichloroethylene was about 110 mg/l milk (the highest predicted concentration was about 220 mg/l). The measured concentration of trichloroacetic acid in milk was about 2.5 mg/l on day 11 and about 6 mg/l on day 14 (the highest predicted concentration was about 9 mg/l).

In a drinking water study of Fisher *et al.*[15], lactating rats were given water containing 333 mg/l *trichloroethylene* for 3 weeks (5 days a week). On day 13, 14 and 21 milk was collected for trichloroethylene and trichloroacetic acid analysis. The concentration of trichloroethylene was below the detection limit, the concentration of trichloroacetic acid in milk was about 0.4, 0.6 and 1.5 mg/l on day 13, 14 and 21, respectively.

2.4 Conclusion

The human studies concerning the potential effects on fertility after exposure to trichloroacetic acid (or trichloroethylene) are difficult to interpret because of a lack of relevant exposure data or the absence of a control groups[5,6].

No animal data are available concerning the effects on fertility of trichloroacetic acid itself. In a study with mice, Forkert *et al.*[8] showed that after inhalatory exposure to a high concentration of trichloroethylene (5370 mg/m^3), trichloroacetic acid is formed in the epididymis. After exposure for four weeks, damage to the epididymal epithelium was observed which affected the normal development of sperm. The committee is of the opinion that this process might also occur in humans. Fertility parameters, however, were not investigated in this mice study. In addition, Manson *et al.*[9] showed no effects on female fertility in Long Evans rats treated by gavage with trichloroethylene (dose levels up to 1000 mg/kg bw/dat. At this dose level, general toxicity (reduced body weight gain) was observed. Zenick *et al.*[10] observed an impaired copulatory behaviour of male Long Evans rats treated orally with trichloroethylene at 1000 mg/kg body weight/day. Nelson and Zenick[11], however, stated that the alterations seen in sexual behaviour might be attributed to the narcotic properties of the compound.

In conclusion, the committee is of the opinion that no conclusions can be drawn based on the human studies on fertility and no animal data is available concerning the effect on fertility after exposure to trichloroacetic acid. Therefore, the committee recommends not classifying trichloroacetic acid with respect to its effects on fertility because of a lack of appropriate data.

The human study of Lindbohm *et al.*[7] did not show an association between maternal occupational exposure to trichloroethylene and spontaneous abortions. No other human studies were available.

Smith *et al.*[12] observed reduced foetal weight and foetal length, soft tissue malformations (mainly in the cardiovascular system), skeletal malformations and eye defects in rats treated by gavage to trichloroacetic acid (330 mg/kg bw/day). These effects were observed in the presence of slight maternal toxicity (increased kidney and spleen weight). At higher dose levels, embryolethality was observed in the presence of decreased maternal weight. The committee is of the opinion that the specific developmental effects observed after exposure to 330 mg trichloroacetic acid/kg bw/day) can not be attributed to the slight maternal toxicity.

Johnson *et al.*[14] found that 291 mg trichloroacetic acid/kg bw/day (in drinking water) increased the post-implantation loss and incidence of cardiac defects. These effects were observed in the absence of maternal toxicity. In a study of Fisher *et al.*[13] treatment with oral dosages of trichloroacetic acid (300 mg/kg body weight/day) did not result in a statistically significant increase in the inci-

dence of heart malformations, on a per foetus or a per litter basis, when compared to controls.

In conclusion, based on the effects found in the study of Smith *et al.*[12] and Johnson *et al.*[14] (increased incidence of cardiac defects and increased post-implantation loss), the committee recommends classifying trichloroacetic acid in category 2 (substances which should be regarded as if they cause developmental toxicity to humans) for its effects on development. In the well-performed study of Fisher *et al.*[13], no effects were observed on the incidence of heart malformations after treatment with trichloroacetic acid. However, these negative results were no reason for the committee to adjust the recommendation classifying in category 2. These differences in study outcomes might have been a result of differences in exposure routes or experimental design. Finally, the committee is of the opinion that a classification in category 2 is supported by the fact that trichloroethylene, of which trichloroacetic acid is a main metabolite, is classified in category 2 as well.

In rats, the concentration of trichloroacetic acid in milk after treatment by inhalation was 2.5 mg/l [15]. In a drinking water study with rats, the concentration of trichloroacetic acid in milk was maximally 1.5 mg/l [15]. No data are available concerning the possible levels of trichloroacetic acid in human breast milk are available. Furthermore, no human and animal data on the effects of lactation are available. Therefore, the committee is of the opinion that a lack of appropriate data precludes assessment of trichloroacetic acid for effects during lactation.

Proposed classification for fertility

A lack of appropriate human and animal data precludes assessment of trichloroacetic acid for labeling for effects on fertility.

Proposed classification for developmental toxicity

Category 2, T;R61

Proposed labelling for effects during lactation

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

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Annexes

Annex **A**

The committee

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  Toxicologist, Institute for Risk Assessment Sciences, Utrecht
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The first draft of the present document was prepared by MM Tegelenbosch-Schouten from the TNO Nutrition and Food Research Institute in Zeist.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2005. The following persons and organisations have commented on the draft document:

- RD Zumwalde,
  National Institute of Occupational Safety and Health (NIOSH), USA
- E González-Fernández
  Ministerio de Trabajo y Asuntos Sociales, Spain.

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 as 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 postnatal-mental or physical development up to and including normal pubertal development.

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

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

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

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

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

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

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of 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:

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

d 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

e on the basis of evidence in humans indicating a risk to babies during the lactational period.

f 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 in animals (inhalation) with trichloroacetic acid or trichloroethylene.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Forkert *et al.* (2002)[8]	Male CD-1 mice (n=6/group)	Exposed for 4 weeks. Trichloroacetic acid measured in urine. Effect on epididymis determined.	1000 ppm trichloro ethylene for 6 h/d, 5 d/w	Not reported	Levels of trichloroacetic acid increased in time. After 4 w exposure sloughing of epithelial cells.

n= number of animals; h= hour; d= day; w= week; bw= body weight; PND= postnatal day; ppm = parts per million

Table 1.2 Fertility studies in animals (gavage) with trichloroacetic acid or trichloroethylene.

Authors	Species	Experimental period/ design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Manson *et al.* (1984)[9]	Female Long Evans rats (n=23/ group)	Treatment (5 d/w) during 2 w premating, and 1 w mating and (7 d/w) during 3 w gestation. Treated females mated with untreated males.	0, 10, 100 and 1000 mg/kg bw trichloroethylene /d gavage	1000 mg/kg bw/d: 1 rat died during premating and bw gain reduced	10, 100 and 1000 mg/kg bw/d: TCE levels > in fat, adrenals and ovaries at end of premating. No effect on length of estrus cycle and fertility
Zenick *et al.* (1984)[10]	Male Long Evans rats (n=10/group)	Treatment for 6 w (5 d/w). At the end of w1 and w5 and 4 w post-exposure males mated with ovariectomized females	0, 10, 100 and 1000 mg/kg bw trichloroethylene/d by gavage	1000 mg/kg bw/d: bw gain reduced (significantly)	1000 mg/kg bw: w1 impaired copulatory behaviour, no effects semen parameters; no effect in w5 and 4w after dosing Remark: Effect on copulatory behaviour probably due to narcotic properties

n= number of animals; h= hour; d= day; w= week; bw= body weight; PND= postnatal day, TCE= trichloroethylene

Table 2.1 Developmental toxicity studies in animals (gavage) with trichloroacetic acid or trichloroethylene.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Manson *et al.* (1984)[9]	Female Long Evans rats (n=23/ group)	Treatment (5 d/w) during 2 w premating, and 1 w mating and (7d/w) during 3 w gestation. Treated females mated with untreated males.	0, 10, 100 and 1000 mg/kg bw trichloroethylene /d gavage	1000 mg/kg bw/d: 2 rats died during pregnancy and 1 rat on the day of delivery. bw gain reduced	1000 mg/kg bw/d: Neonatal survival < in high dose group (mainly of female offspring)
Smith *et al.* (1989)[12]	Female Long Evans rats (n=20-26 females/ group)	Female Long Evans rats treated by gavage on d 6-15 of gestation Treatment: orally by gavage	0, 330, 800, 1200 and 1800 mg trichloroacetic acid/kg bw/ d	≥ 800 mg/kg bw/ d: reduced maternal bw gain ≥ 330 mg/kg bw/ d: increased spleen and kidney weight	800 mg/kg bw/d: embryolethality ≥ 330 mg/kg bw/d decrease foetal weight and foetal length ≥ 330 mg/kg bw/d: soft tissue malformations; mainly in the cardiovascular system ≥ 1200 mg/kg bw/d skeletal malformations ≥ 800 mg/kg bw/d microphthalmia and anophthalmia

Fisher *et al.* (2001)[13]	Female Sprague Dawley rats (n=19-25/ group)	Treatment from gestation d 6-15; sacrifice gestation d 21 Treatment: orally by gavage	500 mg/kg bw trichloro-ethylene/d; 300 mg/kg bw trichloroacetic acid/d; 300 mg/kg bw dichloroacetic acid/d	bw gain during pregnancy reduced in TCA and DCA groups; Reduced uterine weight in TCA-treated females	- Foetal body weight reduced in TCA and DCA-treated groups - No increased heart malforma-tions per litter or foetus

n= number of animals; h= hour; d= day; w= week; bw= body weight; PND= postnatal day
TCE= trichloroethylene TCA= trichloroacetic acid DCA= dichloroacetic acid

Table 2.2 Developmental toxicity studies in animals (drinking water) with trichloroacetic acid or trichloroethylene.

Authors	Species	Experimental period/design	Dose	General toxicity	Effects on reproductive organs/ effects on reproduction
Johnson *et al.* (1998)[14]	Female Sprague Dawley rats (n=8-55)	Treatment during gestation. Sacrifice on gesta-tion d 22	2730 ppm TCA in drinking water (corresponding to 291 mg/kg bw/d) and other metabo-lites of TCE in various concentra-tions	No effects observed	2730 ppm TCA: significantly increased post-implantation loss and significant increase in car-diac defects

n= number of animals; h= hour; d= day; w= week; bw= body weight; PND= postnatal day; ppm = parts per million
TCE= trichloroethylene TCA= trichloroacetic acid DCA= dichloroacetic acid