β-Chloroprene

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 'β-Chloroprene'
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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 β-chloropreen. 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,

prof. dr JA Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 340 7520
E-mail: A.vd.burght@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

β-Chloroprene

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. 2003/06OSH, 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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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondheidsraad 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 β-chloropreen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit is de commissie van mening dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat β-chloropreen de fertiliteit niet schaadt. Daarom adviseert de commissie β-chloropreen niet te classificeren.
- Voor ontwikkelingsstoornissen is de commissie van mening dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat β-chloropreen de ontwikkeling van het nageslacht niet schaadt. Daarom adviseert de commissie β-chloropreen niet te classificeren.
- Voor effecten tijdens lactatie adviseert de commissie om β-chloropreen niet te kenmerken wegens onvoldoende 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 β-chloroprene.

The committee's recommendations are:

- For effects on fertility, the committee is of the opinion that a lack of appropriate human data precludes the assement of ß-chloroprene for fertility and sufficient animal data show that no classification for effects on fertility is indicated.
- For developmental toxicity, the committee is of the opinion that a lack of appropriate human data precludes the assement of ß-chloroprene for effects on development and sufficient animal data show that no classification of β-chloroprene is indicated.
- For effects during lactation, the committee is of the opinion that due to the lack of appropriate data β-chloroprene 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 β-chloroprene 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 J Krüse and dr JAGM van Raaij of the OpdenKamp Registration & Notification, The Hague, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The proposed 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 online 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.

Human studies on β-chloroprene regarding its effects on fertility and development are described in the text and summarised in Annex D. 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 characterisation, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter **2**

β-Chloroprene

2.1 Introduction

Name	:	β-chloroprene
synonyms	:	2-chloro-1,3-butadiene, 2-chlorobuta-1,3-diene, 2-chlorobutadiene, chlorobutadiene, chloroprene, β-chloroprene
CAS-no	:	126-99-8
EINECS no	:	240-818-0
Examples of use	:	as chemical intermediate in the production of polychloroprene (neoprene) elastomer
Mol weight	:	88.5
Molecular formula	:	C_4H_5Cl
Chem formula	:	CH_2=CCl-CH=CH_2
Conversion factor	:	1 ppm = 3.68 mg/m^3 at 760 mm Hg and 20^oC 1 mg/m^3 = 0,272 ppm

β-Chloroprene readily polymerizes when exposed to light and heat and can oxidize to form peroxides, acids and other products. In some studies, β-chloroprene vapour generation was achieved by bubbling air through chloroprene, but the methods used to prepare and generate the concentrations of β-chloroprene for inhalation studies are often not specified. Therefore many signs of toxicity reported in the literature may be due to the fact that the test animals were exposed to reaction products of β-chloroprene such as

dimers and peroxides. This may explain the observed inconsistencies between the results of different research groups (Cul78). Moreover, due to its reactivity β-chloroprene should be stored at 0ºC or below under nitrogen and may contain significant quantities of inhibitors.

2.2 Human studies

Koëter *et al* reviewed several Russian studies (Koë89). In these studies, menstrual disorders, increased number of miscarriages and functional disturbances in spermatogenesis were described. Barlow *et al* (Bar82) cited a study concerning male workers in a polychloroprene factory in France. One hundred of 130 workers exposed to chloroprene showed symptoms of gross over-exposure (chemical burns). In addition, in some of these workers conjunctivitis, hair loss and sexual impotence, involving both libido and sexual dynamics, were observed. (Bar82). Sanotskii (San76) cited a Russian study with 143 workers in β-chloroprene shops. Functional disturbances in spermatogenesis and morphological abnormalities of sperm were described. In addition, an increased number of spontaneous abortions in the wives of β-chloroprene exposed workers was found

The committee concluded that data on the human toxicity of β-chloroprene are scarce and that the quality of the available studies is limited. Moreover, it is possible that effects observed may be related to exposure to other compounds than β-chloroprene, since it is often used in a (rubber) industrial environment with exposure to several chemicals. This might explain the large differences between the no effect levels in studies from East and West-Europe. However, in none of the human studies the exposure was measured or specified.

Fertility

No publications were found concerning effects of pure β-chloroprene on human fertility.

Developmental studies

No publications were found concerning effects of pure β-chloroprene on human development.

Lactation

No publications were found concerning the effects on lactation and the excretion of pure β-chloroprene in human breast milk.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarise the fertility and developmental toxicity studies with β-chloroprene in experimental animals.

Fertility

In two dominant lethal tests, male C57BL/6 mice were exposed to 0, 0.064, 0.32 or 3.5 mg/m^3 β-chloroprene (14-15/group) or to 0, 0.05, 0.13 and 1.85 mg/m^3 β-chloroprene (8-11/group) by inhalation for two months. After exposure the males were mated to 2 or 3 untreated females. In both experiments, fertility (expressed as fertilizing ability) was unaffected. In the first experiment, the total embryonic mortality increased from 29% in the controls to 52, 50 and 63% at 0.064, 0.32 and 3.5 mg/m^3, respectively. In the second experiment, embryonic mortality increased from 19% in the controls to 33, 36 and 42% at 0.05, 0.13 and 1.85 mg/m^3 respectively (San76).

In a dominant lethal study of Sanotskii (San76), male white rats (10/group; strain not specified) were exposed to 0, 0.057 and 0.14 mg/m^3 β-chloroprene for 2.5 months (further specification of exposure not given) and thereupon mated to untreated females. The embryonic death rate was increased from 10% in the controls and the 0.057 mg/m^3 group to 21% in the highest dose group. The majority of the deaths occurred after implantation.

In two studies of Immel and Willems (Imm78a, Imm78b), male Wistar rats (12/group) were exposed to 0 and 180 mg/m^3 or to 0 and 360 mg/m^3 β-chloroprene by inhalation for 6 hours/day during 5 days. Thereafter, they were mated with 2 untreated females/week during 8 weeks. Females were sacrificed 15 days after the mid-week of their mating with the males, and checked for pregnancy. No mortality or abnormalities of condition or behaviour were observed in the males during the exposure period and the eight weeks thereafter. However, during the exposure period the control group did not gain weight and the exposed rats lost weight. The number of corpora lutea, and live and dead implants were determined. In the survivors, no effects on fertility or on mortality of embryos and foetuses were observed.

In a similar study by Immel and Willems (Imm78c), Swiss male mice (12/group) were exposed to 0, 36 and 360 mg/m^3 β-chloroprene for 6 hours/day and 5 days/week during two weeks. Subsequently, they were paired to untreated females during 8 weeks. At the highest dose, 8 of the 12 males died during the first three days of exposure. In the survivors, no effects on fertility or on mortality of embryos and foetuses were observed.

Immel and Willems (Imm79) exposed male rats (5/group) to 0, 36, 120 and 360 mg/m^3 (0, 10, 33 and 100 ppm) β-chloroprene by inhalation for 6 hours/day, 5 days/

week during 3 or 6 months. No dose related changes in sperm concentration or morphological abnormalities were found. However, evaluation of the study is difficult, because of the small size of the treatment groups and the wide variations in different types of abnormalities observed within and between the treatment groups (Imm79).

Mice (C75BL/6, 7-8/dose), that were exposed to 0.06, 0.32, and 3.5 mg/m^3 β-chloroprene during two months, showed adverse effects on the spermatogenesis by an increase in the number of tubules with desquamating germinal epithelium at the two highest doses. The effect was dose-related. However, neither the spermatogenesis index, nor the total number of spermatogonia were affected at these dose levels (San76).

Appelman and Dreef-van der Meulen studied the effects of β-chloroprene on male and female reproduction in a two-generation study in rats (App79). Male Wistar rats (F0, 25/group) were exposed to 0, 36, 120, and 360 mg/m^3 β-chloroprene by inhalation for 6 hours/day, 5 days/week during 13 weeks. Thereupon, they were mated with untreated females. No effects on the fertility of the males of the F0-generation were observed, nor on the intra-uterine mortality and the litter size. Microscopic examination of the testicles did not show any abnormality. However, a reduction in body weight gain was observed in the highest dose group. Male and female animals from the F1-generation (20/sex/group) were exposed to the same levels as the F0-generation during 10 weeks from week 4 after birth. No adverse effects with respect to intrauterine death and litter size were observed. Again reduction in body weight gain (growth retardation) was observed in the medium and high dose groups. The relative weights of liver and ovaries in the female rats were elevated after exposure to 360 mg/m^3. No effects on postnatal mortality and general condition of the F1-generation were observed.

In a similar two-generation study, female rats (F0, 25/group) were exposed to 0, 36, 120, and 360 mg/m^3 β-chloroprene (6 hours/day, 5 days/week) during 13 weeks. Thereupon they were mated with untreated males. The offspring was again exposed during 10 weeks. No adverse effects with respect to intrauterine death, litter size, postnatal death and general condition of the offspring were observed. In the F0-generation, a decrease in body weight gain was observed in the highest dose group and in the F1-generation both at 119 and 360 mg/m^3. In the F1-generation, the reduction in growth was more pronounced than in the F0-generation, but this was attributed to a difference in diet between the two generations. The β-chloroprene used in these studies was freshly purified (App79).

In another study, male rats (Charles River-CD, 5/group) were exposed to 0 and 90 mg/m^3 of β-chloroprene, 4 hours daily for 22 consecutive days. Thereupon they were mated with untreated virgin females (3 new females/male each week) for 8 consecutive weeks. Mated females were allowed to deliver and raise their pups to weaning. The results showed that the reproductive capability of the male rats was not affected, as no adverse effects were observed on fertility, litter size and postnatal survival of the

offspring. The mating index, number of pups/litter, the viability index and the lactation index were not affected. The β-chloroprene used in the study had a purity of 99.9% and contained less than 50 ppm dimers (Cul78).

F344/N rats (10/group/sex) were exposed to β-chloroprene by inhalation at concentrations of 0, 5, 12, 32, 80 and 200 ppm for slightly more than 6 hours/day, 5 days/week for 13 weeks. Sperm motility and vaginal cytology evaluations were performed on all rats exposed at 0, 5, 32, and 200 ppm (0, 18, 115 and 720 mg/m^3) β-chloroprene. Reproductive parameters evaluated included testicular weight, epididymal weight, caudal weight, sperm count, sperm motility, and sperm morphology in males and oestrual cyclicity in females. Complete necropsy was performed on all animals, 24 hours after the last exposure. One male rat in the 200 ppm (720 mg/m^3) group died on the second day of exposure, but no other early mortalities occurred. No effects on body weights of males and females were observed. Clinical findings in the 200 ppm (720 mg/m^3) males included red or clear discharge around the nose and eyes. An exposure-related increase in mean absolute kidney weight was observed in males at 200 ppm (720 mg/m^3) and in females at the two highest dose levels, but this increase was not associated with histopathological changes in the kidney. In the rats, exposure to 80 ppm chloroprene or higher caused degeneration and metaplasia of the olfactory epithelium. Exposure to 200 ppm (720 mg/m^3) β-chloroprene caused anemia, hepatocellular necrosis and a significant reduction in sperm motility, 80.0% vs. 86.7% in the controls. No atrophy of the testes was observed. β-Chloroprene did not affect other male reproductive parameters or interfere with oestrual cyclicity in females (Mel96).

B6C3F1 mice (10/group/sex) were also exposed in this study in the same way as the rats. In contrast to the rat study, the 200 ppm (720 mg/m^3) exposure level was not tested since all mice died in a two week pilot exposure at this level. Sperm motility and vaginal cytology evaluations were performed at 0, 12, 32 and 80 ppm (43, 115, 288 mg/m^3) β-chloroprene. During the exposure period no deaths were observed. Body weight gain in the highest exposure group was slightly lower than that of the controls. No exposure related effects were observed in organ weights (including testes), haematology or clinical chemistry parameters. No atrophy of the testes was observed. The exposure did not affect the male reproductive parameters or alter the oestrual cyclicity in females. The purity of the ß-chloroprene used in this study was greater than 97.9 % and contained less than 0.2 % chloroprene dimers. The peroxide content was less than 0.2 mequivalent/kg chloroprene (Mel96).

Development

In two studies by Culik *et al* (Cul 78), pregnant rats were exposed to 0, 3.6, 36 and 90 mg/m^3 β-chloroprene by inhalation for 4 hours daily, using two different exposure

protocols. In the first study, dams (43-48/group) were exposed on days 1-12 and sacrificed on day 17 to evaluate the embryotoxic potential of β-chloroprene. In a second teratology study, dams (19-24/group) were exposed on days 3-20 of gestation and sacrificed on day 21. In both studies, no maternal, embryonal or foetal toxicity was observed. Litter size, average numbers of implantation sites per litter, and pre-implantation losses among exposed females did not differ significantly from the controls. Only in the teratology study at an exposure level of 36 mg/m^3, a slight but statistically significant increase in the number of dams with resorptions was observed. However, this was judged not to be of toxicological significance, as it was not observed at 90 mg/m^3 or in the embryotoxicity study. In the teratology study, there was a slight but statistically significant increase in the average body weight of the foetuses of dams exposed to 90 mg/m^3. Foetuses from dams exposed to 36 and 90 mg/m^3 showed a significantly increased crown-rump length. Also a significant increase in foetal body weight was observed at he highest dose. No major external, skeletal or soft tissue malformations were seen. The committee concluded that neither developmental toxicity nor maternal toxicity was observed in this study. Therefore, these results were considered inconclusive (Cul78).

Koëter and Appelman (Koë80) exposed pregnant Wistar rats to 0, 36, 90, 270 and 360 mg/m^3 β-chloroprene by inhalation for 6 hours/day. In a preliminary study (7 animals/group), the rats were exposed on gestation days 6-16, whereas in an extended study with 30 animals/group, exposure occurred at days 4-16 of gestation. The animals were sacrificed on day 21 of gestation and evaluated for maternal toxicity, embryotoxicity and teratogenicity. In the extended study, the foetuses were examined for visceral and skeletal effects. At the three highest doses reduction in growth and food intake was observed. Apart from some foetal growth depression, which was not considered to be biologically significant, no signs of embryotoxicity or teratogenicity were observed.

In an inhalation study, the potential of β-chloroprene to cause developmental toxicity in New Zealand white rabbits following gestational exposure was investigated. The rabbits were exposed to 0, 37, 147, 644 mg/m^3 (0, 10, 40 and 175 ppm) β-chloroprene vapours, 6 hours/day, 7 days/week. Each treatment group consisted of approximately 15 artificially inseminated females exposed on day 6 through day 28 of gestation. Body weights were measured throughout the study period, and uterine and fetal body weights were obtained on day 29 of gestation. Implants were enumerated and their status recorded and live foetuses were examined for gross, visceral, skeletal, and soft-tissue craniofacial defects. There were no overt signs of maternal toxicity and the maternal body weight gain was not affected. Exposure of the pregnant rabbits to β-chloroprene had no effect on the number of implantations, the mean percentage of live pups per litter, or on the incidence of resorptions per litter. Foetal body, kidney and liver

weights (as means of litter means) were not affected, nor the foetal sex ratio. The incidence of foetal malformations was not increased by exposure to β-chloroprene. No significant alterations in the incidence of total foetal variations or reduced ossifications were observed among the exposed groups. The results of the study indicate that gestational exposure of New Zealand white rabbits to 10, 40, and 175 ppm β-chloroprene did not result in observable toxicity to either the dams or the offspring (Mas94). The committee concluded that neither developmental toxicity nor maternal toxicity was observed in this study. Therefore, these results were considered inconclusive.

Lactation

No animal studies concerning effects on lactation were available.

2.4 Overall conclusions

Sanotskii (San76), Barlow and Sullivan (Bar82) and Koëter *et al* (Koë89), cited and reviewed a number of East European studies in which the effects of β-chloroprene on fertility and development were studied. The Dutch Expert Committee on Occupational Standards (DECOS) (DEC93), a committee of the Health Council, recommended a health-based occupational exposure limit for β-chloroprene based on these East European studies. They concluded that there were weak indications for disturbance of sexual functions in men and women and for a negative influence on the pregnancy (increased number of abortions) after exposure of male workers to β-chloroprene. However, the present committee is of the opinion that due to the lack of experimental details, it is difficult to evaluate the outcomes of these studies properly. Especially information on the exposure levels in human studies and the purity of the β-chloroprene used in these studies is insufficient for a adequate evaluation of the results. Moreover, in several papers (e.g. Cul78, Bar82, Koë80), it is suggested that due to the presence of impurities in the test compound, these studies show a much higher toxicity than studies in which precautions are taken to avoid the presence of contaminants. Therefore, the committee is of the opinion that a lack of appropriate human data precludes the assessment of β-chloroprene for effects on fertility and development.

In studies with animals in which the purity of the test compound was well-controlled, no biologically significant effects on fertility were observed in the absence of general toxic effects (Cul78, App79, Mel96). Therefore, the committee is of the opinion that the animal data show that no classification is indicated for effects on fertility.

In studies with animals, no effects were observed on development by Culik *et al* and Mast *et al* (Cul78, Mas94). However, no maternal effects were observed either and therefore the committee considers these results as inconclusive. Koëter *et al* did not observe effects on development at doses which caused maternal toxicity (Koë80). Therefore, based on the study of Koëter *et al*, the committee is of the opinion that sufficient animal data show that no classification is indicated for effects on development.

No publications concerning the excretion of ß-chloroprene in human or animal milk were available. Therefore, a lack of appropriate data precludes the assessment of β-chloroprene for labelling for effects during lactation.

Proposed classification for fertility

A lack of appropriate human data precludes the assessment of β-chloroprene for fertility and sufficient animal data show that no classification for effects on fertility is indicated.

Proposed classification for developmental toxicity

A lack of appropriate human data precludes the assement of β-chloroprene for developmental toxicity and sufficient animal data show that no classification for effects on development is indicated.

Proposed labelling for effects during lactation

Lack of appropriate data precludes assessment of β-chloroprene for labelling for effects during lactation.

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A The committee

B Comments on the public draft

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

D Fertility and developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist, Institute for Risk Assessment Sciences, Utrecht
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, Den Haag
- HFP Joosten
  Toxicologist, NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  professor of Medical Genetics, paediatrician, UMC, Utrecht
- JHJ Copius Peereboom-Stegeman
  Toxicologist, Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist, National Institute of Public Health and the Environment, Bilthoven
- N Roeleveld
  Epidemiologist, Catholic University Nijmegen, Nijmegen
- DH Waalkens-Berendsen
  Reproductive toxicologist, TNO Nutrition and Food Research, Zeist
- PJJM Weterings
  Toxicologist, Weterings Consultancy BV, Rosmalen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by K Kruse and JAGM van Raaij of OpdenKamp Registration and Notification, the Hague, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: RA Aksel.
Lay-out: M Javanmardi/J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2002 for public review. No persons and organisations have commented on the draft review.

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, peri/postnatal defects, and impaired postnatal, mental or physical development up to and including normal pubertal development.

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

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

Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

See tables on the next pages.

Table 1.1 Fertility studies with b-chloroprene in animals.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs/effects on reproduction	remarks
San76	C57BL/ 6 mice 8-11 males/ group	Dominant lethal test. Two months exposure and then mating to untreated females.	0, 0.05,0.13, 1.85 mg/m³ (inh)		Fertility unaffected. Increase in total embryonic mortality from 19% (controls) to 33, 36 and 42% at 0.05, 0.13 and 1.85 mg/m3, respectively	Validity of the results concerning the pre- and post-implantation losses questionable due to limitations of the experimental methods.
San76	C57BL/ 6 mice 14-15 males/ group	Dominant lethal test. Two months exposure and then mating to untreated females.	0, 0.064,0.32, 3.5 mg/m³ (inh)		Fertility unaffected. Increase in total embryonic mortality from 29% (controls) to 52, 50 and 63% at 0.064, 0.32 and 3.5 mg/m³, respectively	Validity of the results concerning the pre- and post-implantation losses is questionable due to limitations of the experimental methods
San76	White rats10 males/ group	Dominant lethal test. 2.5 months exposure and then mating to untreated females.	0, 0.057, 0.14 mg/m³ (inh)		The embryonic death rate was increased from 10% at 0 and 0.057 mg/m³ to 21% at 0.14 mg/m³. The majority of deaths occurred after implantation.	
Imm78a Imm78b	Wistar rats 12 males/ group	6 hours/day for 5 days. Thereafter mating with 2 untreated females/ week for 8 weeks. Sacrifice 15 days after mid-week of mating	0, 180, 360 mg/ m³ (inh)		No adverse effects on fertility (pregnancy, numbers of corpora lutea, live and dead implants. No effect on survival of the offspring	β-Chloroprene freshly distilled under nitrogen
Imm78c	Swiss mice 12 males/ group	2 weeks, 6 hours/ day, 5 days/week. Thereafter mating with untreated females for 8 weeks.	0, 36, 360 mg/ m³ (inh)	At 360 mg/m³ 8/12 males died during first 3 days of exposure	In survivors no effects on fertility or embryomortality	One male from the highest dose group withdrawn from study due to poor reproductive performance β-Chloroprene freshly distilled under nitrogen

Table 1.2 Fertility studies with ß-chloroprene in animals.

authors	species	experimental period/design	dose and route	general toxicity	effects on reproductive organs/effects on reproduction	remarks
Imm79	Wistar rats/ group	3 or 6 months, 6 hours/day, 5 days/ week	0, 10, 33, 100 ppm (inh)		No dose related changes in sperm concentration or morphological abnormalities	
Cul78	Charles River-CD rats. 5 males/ group	22 consecutive days, 4 hours/day Thereupon mating with untreated females (3 females/ male/week) during 8 weeks	0, 90 mg/m³ (inh)		No adverse effects on fertility, litter size and postnatal survival of the offspring	The β-chloroprene in the study had a purity of 99.9% and contained less than 50 ppm dimers.
App79	Wistar rats 25 females/ group	2-generation study, 13 weeks, 6 hours/ day, 5 days/week.	0, 36, 119, 360 mg/m³ (inh)		No effects on fertility in both generations	The β-chloroprene in this study was freshly purified
App79	Wistar rats F0 generation: 25 males/ group.	2-generation study, F0 generation: 13 weeks, 6 hours/day, 5 days/week. Thereupon mating with untreated females.	0, 36, 119, 360 mg/m³ (inh)	F0 generation At 360 mg/m³ reduction in body weight gain	F0 generation: no effects on fertility of F0 males. No effects on intra-uterine mortality and litter size. No histopathological abnormalities in testicles	The β-chloroprene in this study was freshly purified.
	F1 generation: 20/sex/group	F1 generation: 10 weeks from week 4 after birth.		F1 generatioin At 119 and 360 mg/ m³ reduction in body weight gain. In females increase in relative weight of liver and ovaries	F1 generation: No effects on intra-uterine mortality, litter size, post natal death and general condition of the offspring.	

Table 1.3 Fertility studies wit ß-chloroprene in animals.

authors	species	experimental period/ design	dose and route	general toxicity	effects on reproductive organs/effects on reproduction	remarks
Mel96	F344/N rats, 10/sex/ group	13 weeks, 6 hours/day, 5 days/week	0, 5, 12, 32, 80, 200 ppm (inh)	One male in the 200 ppm dose group died on day 2 of exposure. Increase in mean kidney weight in males (200 ppm) and females (80, 200 ppm). At 80 and 200 ppm degeneration and metaplasia of the olfactory epithelium. At 200 ppm anemia and hepatocellular necrosis.	At 200 ppm a significant reduction in sperm motility. No atrophy of the testes. No effects on male reproductive parameters. No interference with oestral cyclicity in females.	The β-chloroprene in the study had a purity of 97.9% and contained less than 0.2% chloroprene dimers. The peroxide content was less than 0.2 mequiv/kg chloroprene
Mel96	B6C3F1 rats 10/sex/ group	13 weeks, 6 hours/day, 5 days/week	0, 5, 12, 32, 80 ppm (inh)	At 80 ppm slightly reduced body weight gain.	No atrophy of the testes. No effects on male reproductive parameters. No interference with oestral cyclicity in females	The β-chloroprene in the study had a purity of 97.9% and contained less than 0.2% chloroprene dimers. The peroxide content was less than 0.2 mequiv/kg chloroprene

Table 2.1 Developmental toxicity studies with β-chloroprene in animals

authors	species	experimental period/ design	dose and route	maternal toxicity	developmental toxicity	remarks
Cul78	Charles River-CD rats 43-48 pregnant females/group	4 hours/ day during GD 1-12, evaluation of embryotoxicity	0 , 3.6, 36, 90 mg/m³ (inh)		No effects on litter size, average number of implantation sites/litter, and preimplantation losses.	
Cul78	Charles River-CD rats19-24 pregnant females/group	4 hours/ day during GD 3-20, evaluation of teratology	0 , 3.6, 36, 90 mg/m³ (inh)		No effects on litter size, average number of implantation sites/litter, and preimplantation losses. At 36 mg/m³ a slight increase in number of dams with resorptions (statistically significant but not tocicological relevant) At 90 mg/m³ a slight increase in average foetal body weight (statistically significant). At 36 and 90 mg/m³ increased crown-rump length (statistically significant). No major external, skeletal or soft tissue malformations.	
Koë80	Wistar rats 7 pregnant females/group	6 hours/ day during GD 6-16	0, 36, 90, 270, 360 mg/m³ (inh)	At 90, 270 and 360 mg/m³ reduction in growth and food intake.	At 270 and 360 mg/m³ some foetal growth depression (not biologically significant). No signs of embryotoxicty or teratogenicity.	
Koë80	Wistar rats 30 pregnant females/group	6 hours/ day during GD 4-16	0, 36, 90, 270, 360 mg/m³ (inh)	At 90, 270 and 360 mg/m³ reduction in growth and food intake.	Visceral and skeletal effects evaluated. At 270 and 360 mg/m³ some foetal growth depression (not biologically significant). No signs of embryotoxicty or teratogenicity.	
Mas94	New Zealand White rabbits15 artificially inseminated females/group	6 hours/ day, 7 days/week during GD 6 -28	0, 10, 40, 175 ppm (inh)	No overt signs of maternal toxicity. No effects on change in maternal body weight	No effect on number of implantations, mean percentage of live pups/litter, or incidence of resorptions/litter. No increase in foetal malformations, total foetal variations or reduced ossifications. No effects on foetal body, kidney and liver weights or on sex ratio.	

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