Methylchloride

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

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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 methylchloride. 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 Staatssecretaris 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

Methylchloride

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. 2004/10OSH, The Hague, September 9, 2004

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Samenvatting

In het voorliggende advies heeft de Gezondheidsraad methylchloride onder de loep genomen. Methylchloride wordt voornamelijk gebruikt bij de productie van silicone polymeren. Daarnaast wordt methylchloride toegepast als oplosmiddel en intermediair bij de synthese van diverse stoffen. 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 methylchloride komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om methylchloride te classificeren in categorie 2 (stoffen die dienen te worden beschouwd alsof zij bij de mens de vruchtbaarheid schaden) en met T;R60 te kenmerken.
- voor effecten op de ontwikkeling adviseert de commissie methylchloride niet te classificeren wegens onvoldoende geschikte gegevens.
- voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om methylchloride te kenmerken.

Executive summary

In the present report the Health Council of the Netherlands reviewed methylchloride. Methylchloride is predominantly used for the production of siliconepolymers. In addition, methylchloride is used as a solvent ans as an intermediate for the synthesis of several chemicals. 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 effects of exposure on lactation are considered.

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

- for effects on fertility, the committee recommends classifying methylchloride in category 2 *(substances which should be regarded as if they impair human fertility)* and to label with T;R60.
- for developmental toxicity, the committee recommends not classifying methylchloride due to a lack of appropriate data.
- the committee is of the opinion that a lack of appropriate data precludes the labelling of methylchloride for effects during lactation.

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 methylchloride by the Health Council's Committee for Compounds toxic to reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by dr ir APM Wolterbeek and ir D.H. Waalkens-Berendsen at the Department of Target Organ Toxicology of TNO Nutrition and Food Research, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Union listed in Annex C.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 2004, 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 breastmilk 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 leads to exceedence of the exposure limit for the general population, eg the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up to 2003 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.

* Organisation for Economic Cooperation and Development

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* 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

Methylchloride

2.1 Introduction

Name	:	Methylchloride
Cas-no	:	74-87-3
Synonyms	:	Chloromethane, monochloromethane
Use	:	Methylchloride is found ubiquitously in nature; the vast majority comes from natural sources as the ocean, microbial fermentation and biomass fires. These sources are thought to exceed anthropogenic sources by at least an order of magnitude, with much of the latter being produced and consumed industrially and not related to the environment. Methylchloride is principally used in the manufacture of methyl silicone polymers and resins and in the manufacture of tetramethyl- and mixed tetramethylethyl-lead antiknock compounds for gasoline. It is used to a lesser extent as a chemical intermediate and as a solvent. Methylchloride is used in the production of methylcellulose, butyl rubber, polystyrene foams, plastics, pharmaceuticals, herbicides, dyes, surfactants and desinfectants. Furthermore, methylchloride was used in thermometric and thermostatic equipment as a refrigerant. Methylchloride was also used to a limited extent as an anesthetic but this application was discontinued due to its toxic side-effects (IARC86, EPA01).
Mol weight	:	50.49
Chemical formula	:	CH_3Cl
Conversion factor	:	1 ppm = 2.064 mg/m^3 (in air at 25°C) 1 mg/m^3 = 0.4845 ppm

Toxicokinetics	:	Methylchloride is readily absorbed from the lungs and is extensively distributed throughout the body. Methylchloride is metabolized by conjugation with glutathione to yield S-methylglutathione, S-methyl-cysteine and other sulfur-containing compounds. These compounds are excreted in the urine or can be further metabolized to methanediol which is metabolized by cytochrome P-450 to yield formaldehyde and formic acid.
R-phrases	:	12-40-48/20 Extremely flammable. Possible risk of irreversible effects. Danger of serious damage to health by prolonged

2.2 Human studies

Fertility

No studies were found regarding the effects of exposure to methylchloride on human fertility.

Developmental studies

No studies were found regarding the effects of exposure to methylchloride on human development.

Lactation

No studies were found regarding the effects of exposure to methylchloride on human lactation.

2.3 Animal studies

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

Fertility

A two-generation reproduction study with Fischer-344 rats was conducted by Hamm *et al.* (Ham85). During the premating period (10 weeks) of the F0-generation, male (40/group) and female (80/group) rats were exposed to methylchloride by inhalation (0, 150, 475 or 1500 ppm; 0, 310, 980, 3096 mg/m^3), 6 h/day for 5 days/week. Subsequently, during the mating period (2 weeks; one male was mated with two females of the same exposure group) the F0-generation male and female animals were exposed 6 h/day for 7

days per week. After the mating period, 10 F0-males per dosing group were sacrificed and the remaining 30 F0-males were removed from exposure and mated with unexposed females. After an additional 10 and 28 weeks recovery period 10 F0-males were sacrificed and the remaining F0-males were mated with unexposed females.

During the gestation and lactation period of the F0-generation, the F0-females were exposed 6 h/day for 7 days per week, up to postnatal day 28 (between gestation day 18 to postnatal day 4 the F0-females were not exposed). During the lactation period (until weaning) F1-generation pups were never directly exposed to methylchloride. After weaning on postnatal day 28, selected F1-generation pups from the 0, 150 and 475 ppm groups were exposed to the same concentrations of methylchloride for 10 weeks and then mated.

During the premating period, body weight gains of both male and female animals exposed to 1500 ppm were statistically significantly decreased (10- 20%) from day 7 (for females) and day 15 (for males). The body weight gains of male and female animals exposed to 475 ppm was significantly decreased up to 5-7% from day 57. The body weight gains in the 150 ppm exposed groups did not statistically decrease. In the male F0-animals of the 1500 ppm group, sacrificed directly after the mating period, exposure-related lesions were observed in the testis (degeneration and atrophy of the seminiferous tubules and the testicular weight was statistically significantly decreased) and epididymis (epididymal sperm granulomas). No difference was observed in the number of exposed or unexposed F0-females that mated with exposed F0-males but no litters were born to exposed or unexposed females mated with males that were exposed to 1500 ppm methylchloride.

When male F0-rats were exposed to 475 ppm and mated with exposed and unexposed female rats, the number of litters born was statistically significantly decreased. No effect on the number of litters born was observed in the 150 ppm group. No effects were observed in both the 150 and 475 ppm groups on litter size, sex ratio, pup viability, pup survival and pup growth. The body weight of the male F0-animals remained statistically significantly decreased when compared to the control group up to week 9 of the recovery period. After a 10 week recovery period, no effect was observed in the number of fertile males in the 475 ppm group as compared to the control group. However, fertility in the 1500 ppm group was only partially recovered (25% versus 75% in the control group). The weight of the testis of the 1500 ppm group was still decreased. Histopathologically, no recovery was observed in the testis but in the epididymis granulomas were no longer observed. Fertility was not improved after another 18-weeks recovery period.

Exposure to methylchloride (150 and 475 ppm) had no statistically significant effect on fertility of the F1-animals, although the animals in the 475 group had fewer litters than the 150 ppm and control groups. The litters in the 475 exposure group had significantly decreased percentages of males and the pup weight gain was significantly less.

Morgan *et al*. (Mor82) described (among others) the histopathological effects in the testis and epididymis of Fischer-344 rats exposed to methylchloride by inhalation (0, 2000, 3500 and 5000 ppm; 0, 4128, 7224, 10320 mg/m^3), 6 h/day for 5 days. Subsequently, the animals were not exposed for 2 days and exposed again for 4 days. On day 5, about 50% of the rats exposed to 5000 ppm methylchloride were killed in extremis. In the 3500 and 5000 ppm group, the rats suffered from diarrhea, incoordination of the fore-limbs and hind-limb paralysis and convulsions. Histopathological lesions were observed in the liver (3500 and 5000 ppm: loss of normal areas of cytoplasmatic basophilia and variable hepatocellular degeneration), kidney (2000, 3500 and 5000 ppm: degeneration of proximal convoluted tubules in the outer stripe of the outer renal medulla), brain (5000 ppm: minimal to moderate degeneration of the cerebellar internal granular layer) and adrenal gland (3500 and 5000 ppm: vacuolar degeneration of the zona fasciculata).

In the testis and epididymis dose-related degenerative changes were observed. The principal changes in the testis were reduced numbers of late stage spermatids with none in severely affected tubules, separation of spermatocytes and early stage spermatids, with sloughing of these cells into the lumen, formation of irregular, apparently membrane bound vacuoles in the germinal epithelium and variable formation of multinucleated giant cells. In severely affected tubules only a thin layer of cells remained attached to the basement membrane. The lumen of the epididymal tubules of animals with testicular lesions contained much reduced numbers of sperm and variable numbers of sloughed spermatocytes and multinucleated giant cells and cellular debris. Eosinophylic, hyaline droplets and degenerating cells of unknown type were present in the epithelium of some tubules.

To characterize testicular and epididymal lesions and any associated effects on reproductive hormones, Chapin *et al.* (Cha84) exposed male F-344 rats to 3500 ppm (7224 mg/m^3) of methylchloride, 6 h/day, for 5 days. Subsequently, the males were not exposed for 3 days and exposed again for an additional 4 days. Males were sacrificed on day 5, 7, 9, 11, 13, 15, 19 and 70 and the testis and epididymis were sampled and processed for histopathological examination. Furthermore, blood was collected for testosterone analysis. The three-day break in exposure was used because of the poor condition of the rats surviving after 5 consecutive days of exposure. Some treated animals showed decreased food consumption (details not presented).

The first consistent testicular lesion was a delay in spermiation, which appeared on day 9 suggesting that late-stage spermatids or Sertoli cells may be the initial targets. Subsequently, germinal epithelial vacuolation and cellular exfoliation became widespread as exposure continued. All animals sacrificed after day 19 also displayed bilateral epididymal granulomas. The nature and distribution of the inflammatory cells indicated that the primary neutrophilic response may be against the tubular epithelium and not

extravasated sperm. After 5 days of exposure, circulating levels of testosterone were reduced from 120 ± 31 ng/ml in controls to less then 6 ng/ml in treated animals. However, methylchloride exposed and control animals responded similarly to challenge with human chorionic hormone (to test Leydig cell function) or with luteinizing hormone releasing hormone (to test pituitary function) suggesting that Leydig cell and gonadotropic function was unaffected by methylchloride treatment.

Eighty male Fischer-344 rats per group were exposed to methylchloride (0, 1000 and 3000 ppm; 0, 2064 and 6192 mg/m^3), 6 h/day for 5 consecutive days (Wor85a, Wor85b). After a 3-day recovery period, 40 males/group were mated with untreated females weekly for 8 weeks (during these weeks the males were not exposed) in order to assess mating performance and dominant lethality (Wor85a). The other 40 males/group were used to study the effect of methylchloride exposure on sperm quality and epididymal and testicular histopathology (Wor85b). In the dominant lethal assay, the males of the 1000 ppm group were sacrificed after the 8-weeks mating period. The males of the control and 3000 ppm treatment group were kept for another additional 8 weeks (during these weeks the males were not exposed) and mated again weekly for 4 weeks with untreated females to assess recovery. Furthermore, in a smaller, additional, second experiment 40 males/group were exposed to methylchloride (0 and 3000 ppm), 6 h/day for 5 weeks and mated with untreated females weekly for 4 weeks and then sacrificed.

No treatment related mortalities were observed. In 30% of the male animals of the 3000 ppm group, sperm granulomas in the epididymis were observed. During the 5-day exposure period, body weights were statistically significantly decreased which recovered within 3 weeks (1000 ppm group) or 4 weeks (3000 ppm) after the exposure period. No effect was observed on mating and fertility in the 1000 ppm group. The number of females mated by males of the 3000 ppm group was statistically significantly decreased only at week 2 postexposure. The number of fertile males of the 3000 ppm group was decreased during the entire study reaching statistical significance at weeks 1, 2 and 3 postexposure. In the 1000 ppm group, no statistically significant effects were observed on the dominant lethal parameters. Exposure of the male rats to 3000 ppm caused a small, but statistically significant, increase in postimplantation loss at week 1 post exposure. Preimplantation loss was statistically significantly increased throughout the 8 week post exposure period.

In the additional second study, similar effects were observed. In week 16 after the recovery period, no effect was observed on postimplantation loss, but preimplantation loss was still, although not statistically significantly, increased. In the companion study into the effects of methylchloride on sperm quality and epididymal and testicular histopathology (Wor85b) no consistent, treatment-related effects were observed in the 1000 ppm group. In the 3000 ppm group, testicular weight was statistically significantly

decreased at week 3 postexposure and declined to less than 50% of control weight at week 8 post exposure. However, by week 16 after exposure testicular weight had recovered to near control levels. In the 3000 ppm group, over 50% of the males had uni- or bilateral granulomas in the cauda epididymis. The testis showed a characteristic cytotoxic response to methylchloride exposure including a delay in spermiation, chromatin margination in round spermatids, epithelial vacuolation, luminal exfoliation and spermatogenic cells and multinucleated giant cells. It was shown that 60 to 70% of the spermatogonial stem cells were killed by methylchloride exposure. The number of sperm cells isolated from the vasa deferentia was statistically significantly decreased and the incidence of sperm cells with an abnormal morphology was increased. Furthermore, sperm motility was decreased. All parameters, except for sperm count, had more or less recovered by the end of the recovery period.

Working *et al.* (Wor86) studied the effect of methylchloride on fertilization. Male Fischer-344 rats were exposed to methylchloride (0, 1000 and 3000 ppm; 0, 2064, 6192 mg/m^3) 6 h/day for 5 consecutive days. After exposure, the males were mated with unexposed females weekly for up to 8 weeks. Twelve hours after mating, females were sacrificed and subsequently, their ova and embryos were sampled and scored as fertilized or unfertilized. Fertilization rate in the control animals was about 88% and was not statistically significantly depressed in the females of the 1000 ppm group (80%). However, in the 3000 ppm group fertilization rate was statistically significantly depressed ranging from 3.4% (in week 2) to 72.3% (in week 8). Paternal toxicity was not described.

In a dominant lethal mutation study of Chellman *et al.* (Che86a), Fischer-344 rats were treated with methylchloride (3000 ppm; 6192 mg/m^3) 6 h/day for 5 days. During the five day exposure period, rats lost weight (data not shown). Body weight loss was still observed at week 1 postexposure, but recovered at week 2 postexposure. No male rats died during the exposure period. No additional general toxic effects were described.

Beginning on day 2 after exposure, each male was mated with one untreated female weekly for 3 weeks. Females were killed 12 days after the end of each respective mating week and examined for pregnancy and dominant lethal parameters. Additional groups of males (n=8) were used for histopathological examination of the epididymis. Statistically significant effects of methylchloride were observed on postimplantation loss (week 1), the number of dead implants per total implants (week 1 and 2), number of pregnant females (week 2), number of implantations (week 2 and 3) and on preimplantation loss (week 2 and 3). Furthermore, histopathological examination showed a widespread infiltration of neutrophils and macrophages into the interstitial tissue of the cauda epididy-

mis of rats treated with methylchloride as well as frequent appearance of inflammatory cells inside the epididymal tubules.

In another study of Chellman *et al.* (1986b), male Fischer-344 rats were exposed to methylchloride (7500 ppm; 15480 mg/m^3) 6 h/day for 2 days. Eight of 12 rats died in the 4 days following exposure. Surviving rats were killed either 1 or 3 weeks post exposure and histopathologically examined for epididymal granulomas that were observed in all surviving rats. In a follow-up study, groups of 5 male rats were exposed to methylchloride (5000 ppm; 10320 mg/m^3) 6 h/day for 5 consecutive days. One animal treated with methylchloride died on the fifth day of exposure. Body weight was statistically significantly decreased on days 3, 4 and 5. Furthermore, tremors, ataxia and fore- and hindlimb paralysis were observed. Relative weights of the kidney, adrenal gland, brain, testis and epididymis were statistically significantly increased. Microscopic examination revealed minimal to severe treatment related lesions in the epididymis, testis, brain, kidney, liver and adrenal gland. These lesions included epididymal sperm granulomas, degeneration of cerebellar granule cells,necrosis of renal proximal convoluted tubules and cloudy swelling of hepatocytes. In the testis methylchloride caused exfoliation of pachytene spermatocytes and early stage spermatids into the tubular lumen, slight separation of early stage spermatids and occasional formation of multinucleated giant cells.

To determine whether the observed cytotoxic effects on sperm cells were the results of methylchloride's effects on the testis or on the epididymis, Chellman *et al*. (Che87) exposed groups of male Fischer-344 rats (18/group) methylchloride (0 or 3000 ppm; 0 or 6192 mg/m^3) 6 h/day for 5 consecutive days. Following exposure, 6 males from each group were killed weekly for 3 weeks for assessment of sperm quality and testicualr and epididymal histopathology. During the exposure period, body weight was decreased as compared to the starting weight. One week postexposure, body weight of the methylchloride treated rats was statistically significantly decreased compared to controls. Two and 3 weeks postexposure, no effect on body weight was observed. No animals died. Relative weight of seminal vesicles (week 1), epididymis (week 2 and 3) and testis (week 3) were statistically significantly decreased. Testicular histopathology was characterized by delayed spermiation, disorganization and destruction of seminiferousepithelium and persistent decreases in the number of mid- to late-stage spermatids. Daily sperm production was statistically significantly decreased during weeks 1-3. In the epididymis uni- or bilateral epididymal sperm granulomas, inflammatory cells, debris and nucleated cells in the tubular lumina of the epididymis. In the vas deferens, sperm numbers were normal at weeks 1 and 2 but by week 3 no sperm cells were detected in the vas deferens of any of the 6 males exposed to methylchloride. Furthermore, the percentage of motile cells was decreased by methylchloride at all three time points, at week 3 there

was no motility at all. Moreover, the incidence of morphologically abnormal sperm was increased.

Developmental toxicity

Mated female Fisher 344 rats (25/group) and female C57BL/6 mice (33 per group) were exposed to methylchloride (0,100, 500 and 1500 ppm [0, 206, 1032 and 3096 mg/m^3]) 6 h/day. Rats were exposed from gestation day 7 to19 and sacrificed on day 20 of gestation. Mice were exposed from gestation day 6 to17 and sacrificed on day 18 of gestation (Wol83a) except for the dams in the 1500 ppm group that were sacrificed in extremis during gestation day 10-14. In rats, no behavioural toxicity was observed at any concentration used but at 1500 ppm maternal food consumption, body weight gain and body weight were statistically significantly decreased. No exposure related effects were observed on implantations, resorptions, dead or live fetuses or sex ratio. In the 1500 ppm group, fetal body weight and female crown-rump length was statistically significantly decreased. Foetopathological examination revealed no exposure-related external and visceral abnormalities. Skeletal examination revealed some evidence of delayed ossification in the 1500 ppm group probably related to the decreased fetal weight.

On the fourth day of exposure mice of the 1500 ppm group displayed urogenital bleeding and central nervous system dysfunction (hunched posture, walking on tip-toes, tremors and imbalance). These mice were prematurely sacrificed between gestation day 10-14. Upon necropsy, these animals showed necrosis of the neurons in the internal granular layer of the cerebellum. In the mice of the other groups, no maternal toxic effects were observed. No reproductive parameters were significantly affected by methylchloride. Fetal body weight and fetal crown-rump length were slightly, but not statistically significantly, increased. No treatment-related external and skeletal effects were observed. Visceral examination revealed small, but statistically significant, increases in the incidence of a heart anomaly in the 500 ppm group. The lesion involved a reduction or absence of the atrioventricular valves, chordae tendineae, and papillary muscles in 6 of 17 litters was distributed between left side (bicuspid or mitral valve) in 3 fetuses and right side (tricuspid valve) in 6 fetuses.

To further explore this effect, mated female C57BL/6 mice (74-77/group) were exposed to methylchloride (0, 250, 500 and 750 ppm [0, 516, 1032 and 1548 mg/m^3]), 6 h/day from gestation day 6 to18 (Wol83b) and sacrificed on gestation day 18. Fetuses were examined for external and visceral abnormalities. From the seventh day of exposure (day 12 of gestation), females exposed to 750 ppm displayed ataxia, tremors, convulsions and hypersensitivity to touch and sound. Six females in this group died and one was killed in extremis. Only the survivors in this group showed a statistically signifi-

cantly decreased body weight gain, body weight on gestation day 18, and a reduced absolute weight gain (weight gain minus gravid uterine weight). No treatment-related effects were observed on pregnancy rate, gravid uterine weight, maternal liver weight, numbers of implantations, resorptions, dead fetuses, live fetuses, sex ratio, mean fetal body weight. A statistically significant and dose-related effect was observed on the incidence of affected or malformed fetuses. All but one malformation (an umbilical hernia in the 250 ppm group) were observed in the heart. A statistically significant increase in the incidence of abnormalities of the heart was observed in the 500 and 750 ppm groups and these included reductions in the number of chordae tendineae and papillary muscles, abnormal tricuspid valve, globular heart, white spots in left ventricle, small right ventricle. No effects were observed at 250 ppm.

John-Greene *et al* (Joh85) questioned the results of Wolkowski *et al* (Wol83a,b). In a similar experiment, John-Greene exposed pregnant C57BL mice on day 11.5 to 12.5 (considering the presence of a copulatory plug as day 0) to 300 ppm methylchloride (600 mg/m^3). In addition, rats were exposed from day 11.5 to 12.0 to 1000 ppm methylchloride. No treatment related heart lesions were observed after 'blind' fetal examinations. The authors concluded that there was a considerable interanimal variability in the appearance of the papillary muscles of the heart. This makes the heart lesions described by Wolkowski *et al* difficult to diagnose.

Lactation

No publications were available.

2.4 Conclusion

No data on the effects of methylchloride on fertility, development and lactation on man were available.

Histopathological studies into the effects of inhalatory methylchloride exposure showed that methylchloride caused degenerative lesions in testis and epididymis of rats and mice (Mor82, Cha84, Ham85, Wor85a, Wor85b, Che86a, Che86b, Che87) mainly characterized by a delay in testicular spermiation and epididymal granulomas. In general, the relative weights of the testis and epididymis were decreased, although in a study of Chellman *et al*. (Che86b), relative testis and epididymis weights were increased. Most probably, the increased relative reproductive organ weights in the latter study were caused by severe body weight loss of the animals exposed to methylchloride (ca. 20%). Sperm motility and sperm concentration were decreased whereas the incidence of abnormal sperm morphology was increased by methylchloride exposure (Wor85b, Che87). Functionally, in dominant lethal assays, it was shown that methyl-

chloride caused an increased postimplantation loss and, in particular, an increased preimplantation loss (Wor85a, Che86a), most probably caused by a cytotoxic response rather than a genotoxic response. Although the described effects on male fertility were mainly achieved at paternally toxic dose levels, recovery experiments showed that the induced effects at higher concentrations were partially irreversible. In the dominant lethal studies of Working *et al*. (Wor85a) and Chellman *et al*. (Che86), post- and preimplantation loss was still increased several weeks after the end of exposure.

Furthermore, in a two-generation study of Hamm *et al*. (Ham85), it was shown that inhalatory exposure to methylchloride reduced fertility and even caused infertility in male animals exposed to 1500 ppm methylchloride for 10 weeks (body weight gain was reduced (10% to 20%) from day 10). After a 28-week recovery period (during which the rats were not exposed), fertility had only partially recovered. After exposure to 475 ppm, the number of litters was reduced, but after a 10 week recovery period, male fertility had recovered. Although the effect on male fertility after exposure to 475 ppm was observed in the presence of a minimal reduction in body weight gain (up to 7%), the committee is of the opinion that this effect could not be explained by the presence of paternal toxicity.

Therefore, based on the study of Hamm *et al* (Ham85), the committee recommends classifying methylchloride in category 2 (‘substances which should be regarded as if they impair fertility in humans’) and labeling methylchloride with R60 (‘may impair fertility’). This conclusion was supported by the studies of Morgan *et al* (Mor82), Chapin *et al* (Cha84) and Working *et al* (Wor85,86), which found comparable effects on fertility at higher dose levels and using a different dosing protocol in the presence of more severe general toxicity.

Wolkowski-Tyl *et al*. (1983a, 1983b) studied the prenatal developmental effects of inhalatory methylchloride exposure in rats and mice. The most striking observation of these studies was a small, but statistically significant and dose-related, effect on the incidence of heart malformations in mice. These malformations consisted of absence or reduction of atrioventricular valves, chordae tendineae and papillary muscles. However, John-Greene *et al*. (Joh85) had several doubts about these findings since these observations were not duplicated in their study in which mice were exposed to methylchloride from gestation day 11.5-12.5 (the critical period for development of the embryonal heart). John-Greene *et al* (Joh85) suggested that the findings observed by Wolkowski-Tyl (Wol83a, Wol83b) may have been an artifact of the section technique or a misdiagnosis. According to Wolkowski-Tyl (Wol85), the inability of John-Greene *et al* (Joh85) to induce these malformations was caused by a different treatment protocol (according to Wolkowski-Tyl, the critical day of embryonal heart development was gestation day 14). Despite this controversy about the effect of methylchloride on prenatal developmental, no more (recent) publications were found concerning the effect of methylchloride on

prenatal development. Therefore, the committee recommends not classifying methylchloride with respect to developmental toxicity because of a lack of appropriate data.

No publications concerning the excretion of methylchloride in human or animal milk were available. Therefore, the committee recommends no labeling of methylchloride for effects during lactation due to a lack of appropriate data.

Proposed classification for fertility

Category 2, T;R60

Proposed classification for developmental toxicity

Lack of appropriate data precludes the assessment of methylchloride for effects on development

Proposed labelling for effects during lactation

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

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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
- JHJ Copius Peereboom-Stegeman
  Toxicologist, University Medical Centre St Radboud, Nijmegen
- HFP Joosten
  Toxicologist, NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  professor of Medical Genetics, paediatrician, University Medical Centre, Utrecht
- AH Piersma
  Reproductive toxicologist, National Institute for Public Health and the Environment, Bilthoven
- N Roeleveld
  Epidemiologist, University Medical Centre St Radboud, 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 Ing. JJA Muller from the RIVM in Bilthoven, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance:
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2004 for public review. The following persons and organisations have commented on the draft document:

- RD Zumwalde,
  Centers for Disease Control and Prevention, NIOSH, USA

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 postnatalmental or physical development up to and including normal pubertal development.

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

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

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

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

Effects on fertility

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

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

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposue is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as 'Toxic to Reproduction', unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

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

b on the basis of results of one or two generation studies in animals which in- dicate the presence of adverse effects on the offspring due to transfer in the milk, and/or

c on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility toxicity studies in animals with methylchloride.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproductive organs/ effects on reproduction	Remarks
Morgan *et al.* (1982)	Fischer-344 rats (10/ sex/ group)	Rats were exposed for 5 days, not exposed for 2 days and exposed again for 4 days. Rats were sacrificed 18 h after the last exposure.	0, 2000, 3500, 5000 ppm (0, 4128, 7224, 10320 mg/m^3) 6 h/day by inhalation.	*5000 ppm*: 50% of animals were killed in extremis. *3500 and 5000 ppm*: Diarrhea, incoordination of the fore-limbs, hind-limb paralysis, convulsions. Lesions in liver (3500 and 5000 ppm), kidney (2000, 3500 and 5000 ppm), brain (5000 ppm) and adrenal gland (3500 and 5000 ppm).	*2000, 3500 and 5000 ppm*: Dose-related testicular and epididymal degeneration.	3 Strains of mice were also exposed but the testis and epididymis of mice were not examined.

Chapin *et al.* (1984)	Fischer-344 rats (2 controls and 6 to 8 treated animals were sacrificed per day)	Rats were exposed for 5 days, non exposed for 3 days and exposed again for 3 days Rats were sacrificed on day 5, 7, 9, 11, 13, 15, 19 and 70.	0 or 3500 ppm (7224 mg/m^3) 6 h/day by inhalation	Some treated animals showed decreased food consumption (details not presented). Pair-fed animals were used as controls. After 5 consecutive days of exposure, the surviving rats were in poor condition.	Delay in spermiation, germinal epithelial vacuolation and cellular exfoliation, bilateral epididymal granulomas. Decreased level of testosterone. Depletion of nonprotein sulfhydril in liver, testis and epididymis.	
Hamm *et al.* (1985)	Fischer-344 rats (40 males and 80 females)	Two-generation reproduction study After mating with exposed females, exposed males were also mated (3 times) with unexposed females.	0, 150, 475, and 1500 ppm (0, 310, 980 and 3096 mg/m^3) 6 h/day for 5 or 7 days/ week by inhalation	*475 and 1500 ppm*: Decreased body weight from day 57 and 10, respectively.	*1500 ppm*: After 10 weeks of exposure: histopathological lesions in testis (degeneration and atrophy of the seminiferous tubules) and epididymis (epididymal sperm granulomas), testis weight decreased, males infertile After 10 and 28 week recovery period: *1500 ppm*: Fertility only partially recovered. Testis weight decreased, testicular lesions (degeneration and atrophy of the seminiferous tubules). *475 ppm*: After 10 weeks of exposure: decreased number of litters. After 10 week recovery period: fertility was completely recovered. F1-generation: *150 ppm*: No effects. *475 ppm*: Trend towards decreased fertility.	
Working *et al.* (1985a)	Fischer-344 rats (40 males per group)	Dominant lethality study: rats were exposed for 5 days. After a 3 day recovery period rats were mated with untreated females weekly for 8 weeks. Supplementary recovery experiments	0, 1000, 3000 ppm (0, 2064, 6192 mg/m^3) 6 h/day by inhalation	No mortalities. *1000 and 3000 ppm*: Decreased body weight that recovered 3 and 4 weeks, respectively, after the exposure stopped.	*1000 ppm*: No effect on mating, fertility and dominant lethal parameters. *3000 ppm*: Epididymal sperm granulomas, reduced fertility, increased postimplantation loss (1 week post exposure) and preimplantation loss (during the entire study)	Combined study with Working *et al.* (1985b)

Working *et al.* (1985b)	Fischer-344 rats (40 males per group)	Study into effects on sperm quality and pathology of testis and epididymis: rats were exposed for 5 days. After a 3 day recovery period rats were mated with untreated females weekly for 8 weeks. Supplementary recovery experiments	0, 1000, 3000 ppm (0, 2064, 6192 mg/m^3) 6 h/day by inhalation	No mortalities. *1000 and 3000 ppm*: Decreased body weight that recovered 3 and 4 weeks, respectively, after the end of exposure.	*1000 ppm*: No treatment-related effects on sperm parameters and histopathology of testis and epididymis. *3000 ppm*: Testis weight decreased, recovered 16 weeks after exposure. Histopathological lesions in testis (delay in spermiation, chromatin margination in round spermatids, epithelial vacuolation, luminal exfoliation and spermatogenic cells and multinucleated giant cells) and epididymis (epididymal sperm granulomas). Decreased concentration and motility, increased abnormal morphology in sperm isolated from vas deferentia; except for sperm count recovery after 16 weeks.	Combined study with Working *et al.* (1985a)
Working *et al.* (1986)	Fischer-344 rats (n=10-20)	Study into effect on fertility: male rats were exposed for 5 days and mated with unexposed females weekly for up to 8 weeks. 12 h after mating ova and embryos were sampled and scored for fertilization.	0, 1000, 3000 ppm (0, 2064, 6192 mg/m^3) 6 h/day by inhalation	Paternal toxicity was not described	Fertilization rate in controls 88%, 1000 ppm 80% and 3000 pppm 3.4-72.3%	.
Chellman *et al.* (1986a)	Fischer-344 rats (n=40, for histopathology n=8)	Dominant lethal assay: treatment for 5 days, 2 days after exposure mating with untreated females weekly for 3 weeks. Additional males were used for histopathology of epididymis	0 or 3000 ppm (0 or 192 mg/m^3) 6 h/day by inhalation	Not described	Increased post- implantation loss (1 week after exposure), dead implants per total implants (week 1 and 2), pregnant females (week 2), number of implantations (week 2 and 3), preimplantation loss (week 2 and 3). Histopathological lesions in epididymis (infiltration of neutrophils and macrophages into the interstitial tissue of the cauda epididymis and inflammatory cells inside the epididymal tubules).	

Chellman *et al.* (1986b)	Fischer-344 rats (n=12)	Treatment for 2 consecutive days. Sacrifice 1 or 3 weeks after exposure for histopathology.	0 or 7500 ppm (0 or 15480 mg/m^3) 6 h/day by inhalation	8 of 12 rats died in the 4 days following exposure	Epididymal granulomas in 4 surviving animals
Chellman *et al.* (1986b)	Fischer-344 rats (n=5)	Treatment for 5 days. Rats were sacrificed immediately after the fifth exposure for histopathology	0 or 5000 ppm (0 or 10320 mg/m^3) 6 h/day for 5 days by inhalation	One rats died on day 5 of exposure. Decreased body weight. Neurotoxicological effects. Histopathological. lesions in and increased relative organ weight of brain, kidney, liver and adrenal gland.	Histopatholical lesions in epididymis (sperm granulomas) and testis (exfoliation of pachytene spermatocytes and early stage spermatids into the tubular lumen, slight separation of early stage spermatids and occasional formation of multinucleated giant cells). Increased relative weight of epididymis and testis.
Chellman *et al.* (1987)	Fischer-344 rats (n=18)	treatment for 5 days. Six rats were killed weekly for 3 weeks for sperm quality and histopathology	0 or 3000 ppm (0 or 6192 mg/m^3) 6 h/day by inhalation.	Decreased body weight. Recovery after 2 and 3 weeks	Relative weight of seminal vesicles, testis and epididymis decreased. Histopathological lesions in testis (delayed spermiation, disorganization and distruction of seminiferous epithelium and decreases in the number of mid- and late stage spermatids) and epididymis (sperm granulomas, inflammatory cells, debris and nucleated cells in the tubular lumina). Decreased daily sperm production, concentration and motility of sperm cells. Increased incidence of abnormal morphology of sperm cells.

Table 2 Developmental toxicity studies in animals with methylchloride.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Developmental toxicity
Wolkowski-Tyl *et al*. (1983a)	Fischer-344 rats (25/group)	Exposure from gestation day 7-19, sacrifice on gestation day 20	0, 100, 500 and 1500 ppm (0, 206, 1032 and 3096 mg/m^3) 6 h/day by inhalation	*1500 ppm*: Decreased food consumption, body weight (gain)	No effects on reproductive parameters. *1500 ppm*: Fetal body weight and crown-rump length decreased. No treatment- related external and visceral observations. *1500 ppm*: Slightly delayed ossification *100 and 500 ppm*: No effects.
Wolkowski-Tyl *et al*. (1983a)	C57BL6 female mice (33/group) mated with C3H male mice to give B6C3F1 fetuses	Exposure from gestation day 6-17, sacrifice on gestation day 18	0, 100, 500 and 1500 ppm (0, 206, 1032 and 3096 mg/m^3) 6 h/day by inhalation	*1500 ppm*: Dams were killed in extremis on gestation day 10-14. On the 4th day of exposure urogenital bleeding and neurotox. effects.	No effects on reproductive parameters. No treatment related external and skeletal observation. *500 ppm*: Increased incidence of heart anomalies. *100 ppm*: No effect
Wolkowski-Tyl *et al*. (1983b)	C57BL6 female mice (74-77 per group) mated with C3H male mice to give B6C3F1 fetuses	Exposure from gestation day 6-17, sacrifice on gestation day 18	0, 250, 500 and 750 ppm (0, 516, 1032 and 1548 mg/m^3) 6 h/day by inhalation.	750 ppm: From the 7th day of exposure neurotox effects. Six females died, one was killed in extremis. Decreased body weight (gain)	No effects on reproductive parameters. No effect on external observations. Dose-related increase in visceral abnormalities, all but one heart abnormalities (statistically significant in 500 and 750 ppm groups). No effect in 250 ppm group

Annex **E**

Abbreviations

Abbreviations used:

bw	body weight
CNS	central nervous system
h	hours
n	number