Chloroform

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr. DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve repro-tox-lijst. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1 of 2 wat betreft effecten op de voortplanting. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In 1996 heb ik hiervoor de Commissie Reproductietoxische stoffen ingesteld.

Hierbij bied ik u - gehoord de Beraadsgroep Gezondheid en Omgeving - de publikatie van de commissie aan over chloroform. Deze publikatie heb ik heden ter kennisname aan de Minister van Volksgezondheid Welzijn en Sport en aan de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer gestuurd.

Hoogachtend,
w.g.
prof. dr JJ Sixma

Chloroform

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. 2000/07OSH, The Hague, 3 May 2000

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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 chloroform onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit, meent de commissie dat er onvoldoende gegevens beschikbaar zijn. Zij adviseert daarom chloroform niet te classificeren.
- Voor ontwikkelingsstoornissen, adviseert de commissie om chloroform in categorie 2 *(Stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken)* te classificeren en met R61 *(kan het ongeboren kind schaden)* te kenmerken.
- Voor effecten tijdens lactatie, adviseert de commissie om chloroform *niet* te kenmerken wegens gebrek aan bewijs.

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 chloroform.

The committee's recommendations are:

- For effects on fertility, the committee recommends no classification of chloroform due to a lack of appropriate data.
- For developmental toxicity, the committee recommends to classify chloroform in category 2 *(Substances which should be regarded as if they developmental toxicity to humans)* and to label chloroform with R61 *(may cause harm to the unborn child)*.
- For effects during lactation, the committee is of the opinion that due to the lack of appropriate data chloroform should *not* be labelled with R64.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulations 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 according to the guidelines of the European Union (Directive 93/21/EEG) by the Health Council's Committee for Compounds Toxic to Reproduction. 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 and 2) of the European Union.

1.2 Committee and procedure

The present document contains the classification of chloroform 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 Ms ir IDH Waalkens-Berendsen, Mr drs WG Blijleven (deceased January 1997) and Ms dr AE Smits-van Prooije at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Community 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 April 1999, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.

- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterization and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a compound as potentially toxic to the breastfed child when exposure to this compound via the milk results in an intake exceeding an exposure limit for the general population, eg the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up to 1995. Literature was selected primarily on the basis of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate by the committee. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft report, the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1998. The results of this search were no reason for the committee to adjust the recommendations.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are indicated.

Animal data are summarised in tables in annex D. Special attention was paid to reversibility of observed effects and data on general toxicity and maternal toxicity.

1.6 Presentation of conclusions

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

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

1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations.

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* for definitions see Tox95

Chapter 2

Chloroform

2.1 Introduction

Chloroform is a colourless and odourless liquid.

Name	:	chloroform
CAS-no	:	67-66-3
Synonym	:	trichloromethane
Use	:	as solvent for fats, oils, waxes, resins; as cleaning agent; in fire extinguishers; as flavouring substance and preservative; in manufacturing of dyes, drugs and pesticides
Mol weight	:	119.39
Chem formula	:	$CHCl_3$

2.2 Human studies

Fertility

The pregnancy outcome of female workers in the pharmaceutical industry was investigated in a case-referent study (Tas90): exposure to organic solvents was associated with the occurrence of spontaneous abortion in a dose-related manner. Chloroform was among these solvents, but individual relationships could not be proven.

Sullivan *et al*. (Sul93) concluded that data available are inadequate to assess the potential of chloroform to induce spontaneous abortions or congenital malformations.

Developmental toxicity

Cardiac defects, neural tube defects, oral clefts and very low birth weight were studied in a case-control study in 4 counties in northern New Jersey (Bov92). Statistically significant associations were found between neural tube defects and concentrations of total trihalomethanes (TTHM) higher than 80 ppb with an odds ratio (OR) of 4.5 and concentrations of total trihalomethanes (TTHM) higher than 15 ppb with an OR of 3.8. For oral clefts, cardiac defects and low birth weight no statistically significant associations were found with TTHM. Mothers of cases and controls were interviewed by telephone; a total of 563 mothers were interviewed.

After adjustment for maternal age, parity, adequacy of prenatal care, marital status, education and maternal smoking, Kramer *et al*. (Kra92) found an association between chloroform levels higher than 10 ppb and an increased prevalence of smaller infants (for the time of gestation) of non-hispanic, white women from Iowa. The major limitations of this study involve the ascertainment and classification of exposures to trihalomethanes.

Bove *et al*. (Bov95) conducted a cross-sectional study using environmental and birth outcome data bases in 4 counties in northern New Jersey where some water supplies were contaminated with total trihalomethane (TTHM). A total of 80,938 live births and 594 foetal deaths were studied. Odds ratios higher than 1.50 were found for the following: TTHM with smaller infants (for the time of gestation), central nervous system defects and oral clefts. TTHM levels greater than 100 ppb reduced birth weight among term births by 70.4 g. The authors stated that this study cannot resolve whether drinking water contaminants caused the adverse birth outcome.

Savitz *et al*. (Sav95) evaluated the risk associated with water source, amount of water ingestion and trihalothanes (THM) concentration using data from-a case-control study of miscarriage, preterm delivery and low-birth weight in North Carolina. The data from the study do not indicate a strong association between chlorination by-products and adverse pregnancy outcome, with possible exception of an increased risk of miscarriage in the highest sextile of THM concentration (adjusted odds ratio= 2.8, 95% interval = 1.1-2.7).

Waller *et al*. (Wal98) examined the exposure to trihalothanes and spontaneous abortion in a prospective study of 5,144 pregnant women in California. Of the four individual trihalothanes, only high bromodichloro methane exposure (consumption of 5 glasses per day of cold tap water containing 18 µg per litre bromodichloro methane) was associated with spontaneous abortion.

Reiff *et al* criticized several of the above mentioned studies because of the lack of exposure data. They were of the opinion that the positive findings should be interpreted cautiously (Rei96). The committee concluded that these studies concern mixtures of chlorination products and/or that human exposures were derived from indirect estimates. This limits the establishment of a causal relation between (the single compound) chloroform and the birth defects that were found. Therefore, the committee is of the opinion that the studies of Kramer *et al*, Bove *et al*, Savitz *et al* and Waller *et al* do not provide sufficient evidence to propose a classification for chloroform.

Lactation

Lechner *et al*. 1988 compared the concentrations of chloroform, carbon tetrachloride and tetrachloroethylene in breast milk of 13 mothers from the surroundings of Innsbruck with breast milk of 20 mothers from Linz, a more industrialized area in Austria (Lec88). No elevation in chlorocarbon levels was detected in either group.

Fisher *et al*. 1997 studied the human blood/air and milk/air partition coefficient in blood and milk samples donated by lactating women (n=9) (Fis97). The objective of this study was to evaluate the potential chemical exposure of a nursing infant by ingestion of contaminated milk from a mother who was occupationally exposed to vapours. To estimate infants' exposure, a generic human pharmacokinetic (PB-PK) lactation model was developed. The model was based on a 8-hour exposure of the mother to a constant vapour concentration equal to the threshold limit value for chloroform (10 ppm) in drinking water. The experimentally determined blood/air and milk/air partition coefficient values were used in the PB-PK lactation model. The predicted amount of chloroform ingested by a nursing infant over a 24-hour period was 0.043 mg. However, this model has not been validated yet and the relevance of this exposure level to the development of the human infant is unknown.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the effects of chloroform on fertility and development in experimental animals.

Fertility

Gulati *et al*. (Gul97, NTP summary) evaluated the effects of chloroform on fertility in CD-1 mice by using a continuous breeding protocol. Chloroform was administered by oral gavage using corn oil as a vehicle. Based on a 14-day dose-response study 8, 20, and 50 mg/kg body weight were chosen to test the effect on fertility and development.

Based on a reference analysis of representative aliquots, it was estimated that the actual doses received were 6.6, 16, and 41 mg/kg bw in the low, mid and high dose groups, respectively. Both male and female mice (20 pairs per treatment group, 40 pairs for control animals) were dosed daily for 7 days prior to and during a 98-day cohabitation period. At the high dose chloroform treatment, body weight was not clearly affected in the parental generation. No effects on fertility were observed.

In 1992, The Nordic Council of Ministers group and the Nordic chemicals control group (Dan92) reviewed the available literature on the toxicology and reproductive hazards of chloroform. It was concluded that data concerning effects of chloroform on fertility and gonadal function in humans, as well as effects on fertility in animals, are either absent or inadequate for the evaluation of effects on fertility and gonadal function.

Developmental toxicity

Inhalation studies

Schwetz *et al*. (Sch74) exposed Sprague-Dawley rats to the subanaesthetic levels of 0, 147, 490 or 1470 mg chloroform/m^3 (0, 30, 100 or 300 ppm) for 7 hours per day on days 6 to 15 of gestation. Exposure to chloroform caused a decrease in the conception rate, a high incidence of foetal resorption (300 ppm), and a retarded foetal development (all dose groups). Examination of the foetuses of dams exposed to 100 ppm revealed a significant incidence of acaudia (absence of tail) or short tails, imperforate anus, subcutaneous edema, missing ribs and delayed ossification of sternebrae. The number of foetuses in the 300 ppm group was too low for a meaningful statistical analysis. Maternal toxicity was observed in the 100 and 300 ppm groups as concluded from anorexia and changes in absolute and/or relative liver weights. At 30 ppm, a slight reduction of maternal body weight gain was observed. True terata (acaudia and imperforate anus) were observed in foetuses of rats exposed to 100 ppm chloroform. These untoward effects were not attributable to anorexia, since the same degree of starvation without exposure to chloroform was not embryo- or foetotoxic. In order to determine whether anorexia was responsible for the embryonic and foetal effects, a second ‘starved’ control group was included in the experiment. The decrease of foetal body weight was not associated with developmental effects in this group.

Dilley *et al*. (Dil77) exposed pregnant rats (number unknown) daily to 20.1 g/m^3 chloroform during days 7 to 14 of gestation. All animals were sacrificed on day 20 of gestation, and the dams and foetuses were examined for gross changes. Chloroform caused increased foetal mortality and decreased foetal weight gain, but no teratogenic effects. Possible maternal toxicity was not described.

Murray *et al*. (Mur79) exposed mice (number unknown) to 0 or 147 mg/m^3 of chloroform for 7 hours/day from day 1 to 8, 6 to 16 or 8 to 16 of gestation. Maternal toxicity was observed among the dams exposed to chloroform from day 6-16 of gestation as a slight decrease in body weight gain, and as significant decrease in body weight gain among the dams exposed from day 1 to 8 and from day 8 to 16. The ability of the females to maintain pregnancy was significantly decreased after exposure to chloroform from day 1 to 8 or 6 to 16 of gestation. A significant increase in the mean number of resorptions per litter was observed among the mice exposed from day 1 to 8. Mean foetal body weight and crown-rump length were decreased significantly among the offspring of mice inhaling chloroform from day 1 to 8 or 8 to 16 of gestation. Cleft palate occurred significantly more often among the litters of mice exposed to chloroform from gestation day 8 to 16. Compared to the control group, the incidence of delayed ossification of skull bones was significantly increased among each of the experimental groups. In addition, delayed ossification of the sternebrae occurred significantly more often among the litters of dams that inhaled chloroform from day 1 to 8 or from day 8 to 16, but not from day 6 to 16 of gestation.

Baeder and Hofmann (1988) exposed mated female Wistar rats by inhalation to 0, 30, 100 or 300 ppm (0, 147, 490 or 1470 mg/m^3) from gestation day 7 to 16 during 7 hours per day (Bae88). In the 0, 30, 100 and 300 ppm treated groups 0, 2, 3 and 8 dams with resorptions only were observed. Foetal weight was decreased in all chloroform treated groups. Decreased maternal body weight and food intake was observed in the 100 and 300 ppm groups.

In a supplementary study, Baeder and Hofmann (1991) exposed mated female Wistar rats by inhalation to 0, 3, 10 or 30 ppm (0, 14.7, 49 or 147 mg/m^3) from gestation day 7 to 16 during 7 hours per day (Bae91). The percentage of small foetuses was statistically significantly increased in the 10 and 30 ppm groups. In the 30 ppm group 1 dam with resorptions only was observed. In this group a slightly reduced food intake was observed as well.

Oral gavage studies

The effect of orally administered chloroform on embryonic and foetal development in the rat was evaluated by Thompson *et al*. (Tho74). Rats received doses of 0, 20, 50 or 126 mg/kg bw/day on days 6-16 of gestation. Body weight gain of dams administered 50 mg/kg bw/day was significantly lower in comparison with controls, and dams from the 126 mg/kg bw/day lost weight. Food consumption was significantly decreased in the 126 mg/kg bw/day group. At 126 mg/kg bw/day statistically significantly decreased body weight of foetuses was observed after administration of chloroform. Teratogenic effects were not observed.

Ruddick *et al*. (Rud83) administered chloroform by gavage to Sprague-Dawley rats from day 6 to 15 of gestation. Chloroform was administered at levels of 100, 200 and 400 mg/kg. Maternal weight gain was depressed in all groups receiving chloroform. Chloroform administration caused decreased maternal haemoglobin and haematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of chloroform. Evidence of a foetotoxic response (not specified) was observed with chloroform, but teratogenic effects were not observed.

The effect of orally administered chloroform on embryonic and foetal development in the rabbit was evaluated by Thompson *et al*. (Tho74). Rabbits received doses of 0, 20, 35 or 50 mg/kg bw/day on days 6-19 of gestation. Body weight gain of dams administered 50 mg/kg bw/day was significantly decreased in comparison to controls. Foetotoxic or teratogenic effects were not observed.

Gulati *et al*. (Gul97, only NTP summary) evaluated the effect of chloroform on fertility and reproduction in the first and second generation of CD-1 mice by use of a continuous breeding protocol at levels of 8, 20, and 50 mg/kg body weight (actual doses were 6.6, 16, and 41 mg/kg bw in the low, mid and high dose groups, respectively). Both male and female mice (20 pairs per treatment group, 40 pairs for control animals) were dosed daily for 7 days prior to and during a 98-day co-habitation period. The offspring from the control and from the high dose group, the F_1 generation, was also evaluated. In the high dose chloroform group, no effects on body weight of either the parents or the offspring were observed. F_1 generation males in the high dose group showed significantly increased epididymal weights. However, the epididymal sperm motility, count and morphology were not affected. F_1 females in the high dose group showed increased liver weight and there were signs of hepatocellular degeneration.

2.4 Conclusion

No relevant data were found concerning the effects of chloroform on human or animal fertility. The potential effect of chloroform as a cause of abortion in humans could not be proven (Tas90). In conclusion, a lack of appropriate data precludes the assessment of chloroform for effects on fertility. Therefore chloroform should not be classified with respect to effects on fertility.

The data concerning the developmental effects in humans (Bov92, Kra92, Bov95, Sav95, Wal98) were insufficient to classify chloroform.

In two inhalatory studies with animals, developmental effects were observed in rats and mice, in the presence of maternal toxicity (Sch74; Mur79). These developmental effects (eg acaudia and imperforate anus) were considered not secondary to the maternal

toxicity. In addition, Baeder and Hofmann (Bae88 and Bae91) observed an increased number of dams with resorptions only, a decreased foetal weight and an increased number of small foetuses at dose levels at which no or only slight maternal toxicity was observed.

In conclusion, the committee is of the opinion that the effects on development were specific (acaudia and imperforate anus in rat (Sch74) and pregnancy maintenance in mice (Mur79)) and could not be explained by maternal toxicity. The studies of Baeder and Hofmann (Bae88 and Bae91) support the findings of Murray and Schwetz (Muy79; Sch74) that inhalatory exposure to chloroform causes an increased number of resorptions.

Therefore, in view of the animal data, the committee recommends to classify chloroform in category 2, substances which should be regarded as if they cause developmental toxicity in humans. Chloroform should be labelled with R61 (may cause harm to the unborn child).

No data were available concerning the transfer of chloroform to human breast milk, but due to its physicochemical properties (Fis97) it may be assumed that chloroform passes to breast milk. However, the committee is of the opinion that this assumption is not sufficient for labelling chloroform for lactation. No data were available concerning effects during lactation.

In conclusion, a lack of appropriate data precludes the labelling of chloroform for effects during lactation. Therefore chloroform is not labelled with respect to effects during lactation.

Proposed classification for effects on fertility

A lack of appropriate data preclude the assessment of chloroform for fertility.

Proposed classification for developmental toxicity

Category 2, R 61.

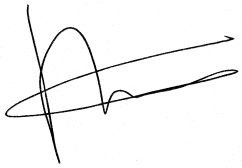
Proposed labelling for effects during lactation

A lack of appropriate data precludes the assessment of chloroform for effects during lactation.

For the committee,
The Hague, 3 May 2000

dr ASAM van der Burght,
scientific secretary

dr BJ Blaauboer,
chairman



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Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
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- JN van den Anker
  Professor of pediatrics and Neonatology; Erasmus University, Rotterdam
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Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. The following organisations and persons have commented on the draft document:

- RJ Millischer, Elf Atochem SA, France.

Annex **C**

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

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear resuts in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:

T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:

T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:

Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 *Comments regarding the categorisation of substances toxic to reproduction*

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1 *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would in-

terfere 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 exposure is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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

Annex **D**

Fertility and developmental toxicity studies

Table 1 Fertility studies with chlorofom.

authors	species	route	experimental period	dose	findings	remarks
Gulati *et al*. (1997)	CD-1 mice	oral (gavage)	continuous breeding protocol[a].	8, 20, 50 mg/kg/bw[b]	Body weight at the high dose chloroform treatment had no apparent effect on either parental (F sub 0) or F sub 1 generation. F sub 1 generation males in the high dose group showed significantly increased epididymal weights. However, the epididymal sperm motility, sperm count and sperm morphology were not affected. F sub 1 females in the high dose group showed increased liver weight and there were signs of hepatocellular degeneration.	Only abstract of the study available.

[a] Both male and female mice (20 pairs per treatment group, 40 pairs for control animals) were dosed daily for 7 days prior to and during a 98-day cohabitation period. The F sub 1 generation from the control and high dose groups were also evaluated.

[b] It was estimated that the actual doses received were 6.6, 16, and 41 mg/kg bw in the low, mid and high dose groups, respectively.

Table 2.1 Developmental toxicity studies with chlorofor.

authors	species	route	experimental period	dose	findings	remarks
Schwetz *et al*. (1974)	Sprague-Dawley rat	inhalation	days 6-15 of gestation for 7 h/day	0, 147, 490 and 1470 mg/m^3	At all dose levels statistically significant decrease in maternal weight gain and food consumption. At 147 mg/m^3 retarded foetal development. At 490 mg/m^3 a significant number of litters containing pups (number unspecified) with missing or shortened tail and imperforate anus, retarded foetal development. At 1470 mg/m^3 the number of litters available for analysis was severely reduced because of a decrease in the rate of conception and a high incidence of foetal resorption. Terata were not observed in this group.	At 1470 mg/m^3 the high level of early embryonic death may mask malformations.
Thompson *et al*. (1974)	Sprague-Dawley rat	oral	days 6-15 of gestation	0, 20, 50, 126 mg/kg bw/day	At 50 and 126 mg/kg bw decreased body weight gain. At 126 mg/kg bw decreased food consumption, maternal kidney and liver damage, and statistically significantly decreased foetal weight. No teratogenic effects.	
Thompson *et al*. (1974)	rabbit	oral	days 6-18 of gestation	0, 20, 35, 50 mg/kg bw/day	At 50 mg/kg bw decreased body weight gain, maternal kidney and liver damage, and statistically significantly decreased foetal weight. No teratogenic effects.	
Dilley *et al*. (1977)	rat (species ?)	inhalation	days 7-14 of gestation for ? h/day	20.1 g/m^3	Increased foetal mortality and decreased foetal weight. No teratogenic effects.	Study was poorly reported.

Table 2.2 Developmental toxicity studies with chloroform.

authors	species	route	experimental period	dose	findings	remarks
Murray *et al*. (1979)	CF-1 mouse	inhalation	days 1-7, 6-15, 8-15 of gestation 7 h/day	0, 147 mg/m^3	Maternal toxicity consisted of a decrease in body weight gain. Also slightly less food and water consumption was observed. In all groups decreased ossification (not significant) Exposure from day 8 to 15 resulted in a significant elevation in the incidence of cleft palate, and significant decreased foetal weight and rump-length. Exposure from day 1 to 7 reduced litter size (increase of resorptions) but no malformations. Decreased mean foetal body weight and crown-rump length were also observed from day 8 to 15.	Cleft palates were seen predominantly in foetuses with retarded growth.
Ruddick *et al*. (1983)	Sprague-Dawley rat	oral	days 6-15 of gestation	0, 100, 200, 400 mg/kg bw/day	Maternal weight gain was depressed in all groups receiving Chloroform. Chloroform administration caused decreased maternal haemoglobin and haematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Chloroform. Evidence of a foetotoxic response (not specified) was observed with Chloroform, but teratogenic effects were not observed.	The observed foetotoxicity was considered to be the result of the maternal toxicity (WGD 1987). Only abstract available.
Baeder and Hoffman (1988)	Wistar rats	inhalation	days 7-16 of gestation for 7h/day	0, 30, 100, 300 ppm	*30 ppm:* 2 dams with only resorptions (vs 0 in control); decreased foetal length *100 ppm*: gestation day 7-17 decreased body weight and food intake, 3 dams with only resorptions, decreased foetal length. *300 ppm*: gestation day 7-17 decreased body weight and food intake; 8 dams with only resorptions; decreased foetal length and weight.	

Table 2.3 Developmental toxicity studies with chloroform

authors	species	route	experimental period	dose	findings	remarks
Baeder and Hoffman (1991)	Wistar rats	inhalation	days 7-16 of gestation for 7h/day	0, 3, 10, 30 ppm	*0 ppm*: no dams with resorptions, 3.2% small foetuses *3 ppm:* 0 dams with only resorptions; 14.2% small foetuses (not statistically significant) *10 ppm*: 0 dams with only resorptions, 24% small foetuses. *30 ppm*: slightly reduced food intake; 1 dams with only resorptions; 26.9% small foetuses.	

Annex **E**

Abbreviations

Abbreviations used:

- body weight
- day
- female(s)
- intraperitoneal
- intravenous
- male(s)
- number
- no adverse effect level
- Organisation for Economic Cooperation and Development
- postnatal