Ethylene oxide

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies over ethyleenoxide
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 2460/AvdB/tvdk/543-E5
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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 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 of 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 ethyleenoxide. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de Minister van Volkgezondheid, Welzijn en Sport en de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Hoogachtend,

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Ethylene oxide

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. 2001/11OSH, The Hague, 20 December 2001

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de 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 ethyleenoxide onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit, adviseert de commissie ethyleenoxide in categorie 2 *(Stoffen die dienen te worden beschouwd alsof zij bij de mens de vruchtbaarheid schaden)* te classificeren en met R60 *(kan de vruchtbaarheid schaden)* te kenmerken.
- Voor ontwikkelingsstoornissen, meent de commissie dat er onvoldoende geschikte humane en diergegevens zijn. Daarom adviseert zij ethyleenoxide niet te classificeren.
- Voor effecten tijdens lactatie, adviseert de commissie om ethyleenoxide niet te kenmerken wegens onvoldoende gegevens.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed ethylene oxide.

The committee's recommendations are:

- For effects on fertility, the committee recommends that ethylene oxide should be classified in category 2 *(Substances which should be regarded as if they impair fertility in humans)* and labelled with R60 (*may impair fertility)*.
- For developmental toxicity, the committee recommends not to classify ethylene oxide due to a lack of appropriate human and animal data.
- For effects during lactation, the committee is of the opinion that due to the lack of appropriate data ethylene oxide should not be labelled.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of ethylene oxide 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 Mrs ir DH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the 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 (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 February 2001, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

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

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).

- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

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

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration is above 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 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. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1999. 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 considered.

Animal data are described in the text and summarised in Annex D.

1.6 Presentation of conclusions

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

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

1.7 Final remark

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

* for definitions see Tox95

Chapter **2**

Ethylene oxide

2.1 Introduction

Name	:	ethylene oxide
CAS-no	:	75-21-8
CAS-name	:	Oxirane
Examples of use	:	as chemical intermediate, as fumigant, as sterilant
Mol weight	:	44.1
Chem formula	:	C_2H_4O
Conversion factor	:	1 ppm = 1.83 mg/m^3 at 760 mm Hg and 20 ^oC

Ethylene oxide is a highly reactive alkylating agent. Ethylene oxide is considered to be a genotoxic carcinogen (WGD89).

2.2 Human studies

Fertility

In an epidemiological study, Joyner (1964 in: ECETOC 1982) examined 37 workers with a continuous occupational exposure of 9-18 mg/m^3 ethylene oxide in air for an average of 10.7 years. Incidences of benign prostatic hypertrophy, acute prostatitis, spermatoceles or seminomas of the testis was not different from a control group

consisting of 41 non-exposed operators, but parameters of reproductive capacity were not studied (Joy64).

Yakubova *et al.* (1976 in: Barlow and Sullivan 1982) reported an increased incidence of gynaecological disorders and abortions in women employed in an ethylene oxide production plant (Yak76). However, the data are not sufficient to determine if these effects were related to the exposure to ethylene oxide.

Hemminki *et al.* (1982) analysed spontaneous abortions in a Finnish hospital sterilising staff using data from a postal questionnaire and a hospital discharge register (Hem82). The data showed that the incidence of spontaneous abortions was 11.3% for the sterilising staff (N=1443) and 10.6% for the nursing auxiliaries (N=1179), who had no exposure to sterilising agents, anaesthetic gases or X-rays. When the pregnancies of the sterilising staff were classified as "exposed" or "unexposed" on the basis of the work history at the beginning of each pregnancy, significantly more spontaneous abortions (16.7%) were observed in the "exposed" than in the "unexposed" pregnancies (6.0%). The excess number of spontaneous abortions was contributed mainly by to pregnancies during which ethylene oxide was used as a sterilising agent. The effect (ie. increased risk of spontaneous abortion) of ethylene oxide exposure remained when the data were controlled for age, parity, decade of pregnancy, consumption of coffee and alcohol and tobacco smoking. In addition, Hemminki *et al.* compared their results with the pregnancy outcome data for sterilising staff and controls identified from Finnisch hospital discharge registers. A significant increase in age-adjusted spontaneous abortion rates was observed for ethylene oxide exposed pregnancies among the sterilising staff (22.6%) compared to the age-adjusted rate among control pregnancies (9.2%). This result confirmed the findings made on the basis of the data obtained from the questionnaires. Hemminki *et al.* (1983) stated, however, that the number of persons in their cohort study was "not large enough to compare abortion rates and known ethylene oxide concentrations" (Hem83). The committee agrees with Hemminki *et al.* (1983) that the cohort in their study was was too small for reliable conclusions. In addition, Gordon and Meinhardt (1983), raised some questions regarding the findings in the study of Hemminki *et al.* (Gor83) as well. They were of the opinion that the information regarding (I) the adjustment for age and parity, and (II) the classification of smoking habits alcohol and coffee consumption were insufficient. In addition, information on whether the duration and degree of these three consumption patterns were accounted for at the time of each pregnancy were considered to be insufficient as well.

Developmental studies

No publications were found concerning developmental toxicity of ethylene oxide.

Lactation

No publications were found concerning ethylene oxide in human breast milk.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarise the fertility and developmental toxicity studies with ethylene oxide in experimental animals.

Fertility

Embree *et al.* (1977) tested the mutagenic potential of ethylene oxide in male germ cells using the dominant lethal assay in rats (Emb77). Male rats were exposed by inhalation to 1800 mg/m^3 ethylene oxide once for 4 hours and mated each week to groups of two females for 10 weeks. A significant increase in foetal death was noted in the exposed group when compared to the negative control group. This significant increase was noted in females mated during the first 5 weeks of the experiment. This corresponds to the residence time of exposed germinal cells after their meiotic division. The authors stated that the significant decrease in the fertility index in week 3 and 4 after ethylene oxide exposure was probably attributable either to a decrease in sexual vigour of the males, to a spermicidal effect of ethylene oxide, or to some other manifestations of the mutagenic properties of the compound. After exposure the following signs of toxicity which were described as slight signs of ethylene oxide toxicity were noted: central depression, diarrhoea, ocular and respiratory irritation. Within 24 hours all rats appeared normal and no deaths occurred.

Mori *et al.* (1991) exposed groups of six male Wistar rats by inhalation to 90, 180, or 450 mg/m^3 ethylene oxide for 6 h per day, 5 days per week for 13 weeks (control group 12 animals) (Mor91). In the 450 mg/m^3 exposure group, epididymal but not testicular weight was reduced. There was slight degeneration in some seminiferous tubules, reduced sperm count in the body and tail but not the head of the epididymis and an increased number of abnormal sperm heads in the tail of the epididymis, mainly due to the presence of immature sperm. An increase in malformed sperm heads, unrelated to dose level, was observed in all treated groups when compared to the control group (15% versus 2%). No effects on the body weight was found in all dose groups.

Female Sprague-Dawley rats (n=39-41) were exposed by inhalation to 0 and 270 mg/m^3 ethylene oxide for 7 h per day, five days per week from 15 days prior to mating until day 16 of gestation (Hac82). The incidence of resorptions was slightly increased. Maternal weight gain and foetal growth were reduced.

The reproductive toxicity of ethylene oxide was evaluated by Snellings *et al.* (1982a) (see also EPA/OTS 1983) who exposed groups of 30 male and 30 female Fischer 344 rats by inhalation to ethylene oxide for (1) 6 h/day, 5 days/week for 12 weeks prior to mating, (2) 7 days/week for 2 weeks cohabitation, (3) 7 days/week for males for 3 weeks after cohabitation and for females gestation days 0-19 and 7 days/week for females and pups 5-21 days postpartum to 0, 18, 59 or 180 mg/m^3 ethylene oxide (Sne82a). Each male was mated with 2 females (1/week), and each female with 2 males (1/week) unless the first mating was successful. There were no F_0 mortalities, adverse clinical effects, or changes in reproductive organs. Body weights and weight gains of the F_0 generation were not significantly affected. The high dose group showed an increased duration of the gestation period, fewer pregnancies/mating period, fewer implantation sites/pregnant female and fewer pups born/implantation site than the control group. Male and female fertility, gestation, gestation survival, 0-4 day survival, 4-14 day survival, and 4-14 day survival indices for treated groups did, however, not significantly differ from controls.

Developmental toxicity

The teratogenic potential of intravenously (iv) administered ethylene oxide was assessed in the CD-1 mouse after treatment with doses of 0, 75 and 150 mg/kg bw at four periods during gestation days 4-6 (period 1), 6-8 (period 2), 8-10 (period 3) and 10-12 (period 4) (LaB80). Maternal animals showed signs of toxicity (19-48% of the animals died) at the 150 mg/kg dose level in periods 1, 3, and 4 but not in period 2. In period 3 and 4, maternal weight gain during treatment was significantly reduced in the 150 mg/kg bw group. Absolute maternal weight gain (weight gained during pregnancy minus uterine weight) was not affected. No maternal toxicity was observed in any period after treatment with 75 mg/kg. A significant reduction in mean foetal body weight compared to controls occurred in all four treatment periods at the 150 mg/kg level, and a significant increase in the percentage of malformed foetuses/litter was observed in periods 2 and 4 at this dose level. Approximately 19% of the foetuses in each litter from maternal animals treated with 150 mg/kg ethylene oxide in period 2 had some type of malformation, predominantly of the cervical and thoracic part of the skeleton.

The reproductive toxicity of ethylene oxide was evaluated by Snellings *et al.* (1982a) (see also EPA/OTS 1983) who exposed groups of 30 male and 30 female Fischer 344 rats by inhalation to ethylene oxide for (1) 6 h/day, 5 days/week for 12 weeks prior to mating, (2) 7 days/week for 2 weeks cohabitation, (3) 7 days/week for males for 3 weeks after cohabitation and for females gestation days 0-19 and 7 days/week for females and pups 5-21 days postpartum to 0, 18, 59 or 180 mg/m^3

ethylene oxide (Sne82a). Each male was mated with 2 females (1/week), and each female with 2 males (1/week) unless the first mating was successful. Pup weight was unaffected, except at day 21, where male neonatal body weight was decreased after exposure to 59 mg/m^3, but not after exposure to 180 mg/m^3.

In a teratology study (Snellings *et al.* 1982b), pregnant Fischer 344 rats (21-22/group) were exposed to ethylene oxide by inhalation at nominal concentrations of 0, 18, 59, or 180 mg/m^3 for 6 h on gestation days 6-15 (Sne82b). The only treatment-related effect noted was a significant decrease at 180 mg/m^3 in male and female foetal body weight relative to foetuses in control groups. No significant differences between treated and control animals were observed with respect to: maternal and foetal survival, number of implantation and resorption sites, number of preimplantation loss, crown-to-rump length, the incidence of external, visceral and skeletal abnormalities. Maternal toxicity was insufficiently examined.

Female Sprague-Dawley rats were exposed by inhalation for 7 h per day, five days per week, on (1) days 7-16 of gestation, (2) on days 1-16 of gestation or (3) 15 days prior to mating and then until day 16 of gestation to 270 mg/m^3 ethylene oxide (Hac82). In the third group the incidence of resorptions was slightly increased. Maternal weight gain and foetal growth were reduced in all groups.

Exposure of rabbits by inhalation to 270 mg/m^3 ethylene oxide for 7 h per day on days 7-19 or 1-19 of gestation resulted in no evidence of maternal toxicity, embryotoxicity or teratogenicity (Hac82).

Generoso *et al.* (1987) demonstrated that exposure of (C3HxC57BL)F_1 or (SECxC57BL)F_1 female mice mated with (C3HxC57BL)F_1 males to ethylene oxide gas (2160 mg/m^3 for 1.5 h) could produce different results depending on the timing of exposure. Females were exposed 1, 6, 9 or 25 h after timed 30-min mating periods. Exposure at 1 or 6 h increased the number of midgestational and late foetal deaths, but these effects were less prominent after exposure at 9 h and none after 25 h. A large number of foetuses that survived after exposure at 6 h had a range of congenital malformations, including omphalocoele, hydropia, thoracoschisis and limb and tail defects. Malformations were also seen in foetuses exposed at 1 h but not in those exposed at 9 or 25 h. Maternal toxicity was suggested to be absent, but not described in detail.

Lactation

No publications were available.

2.4 Overall conclusions

No reliable data concerning effects on fertility in man were available (Joy 64, Yak76, Hem82). Studies in which relatively small groups of women were exposed to ethylene oxide, suggest an increased rate of abortions, but either the data were inadequate (Yak76), or the cohort size was too small for reliable conclusions (Hem82).

Toxic effects on rat sperm cells were observed after exposure to ethylene oxide by inhalation (Mor91). In a study of Snellings *et al.* (Sne82a) the duration of the gestation period was increased and the number of implantations and the number of pups born per implantation were decreased after inhalatory exposure of rats to ethylene oxide in the absence of general toxicity. The effects observed were most probably caused by the dominant lethal effect of ethylene oxide. Furthermore, in another inhalatory study (Hac82) in rats, with exposure to ethylene oxide during the premating and gestation period, a slight increase in the number of resorptions was observed. Dominant lethal effects were observed in male rats in the first 5 weeks of the experiment, corresponding to the residence time of germinal cells after their meiotic division (Emb77).

In conclusion, in view of the animal data the committee proposes to classify ethylene oxide in category 2 (substances which should be regarded as if they impair fertility in humans) and to label the compound with R60 (May impair fertility).

No data were found concerning developmental effects in man after exposure to ethylene oxide.

Intravenous administration of ethylene oxide to mice caused an increased number of resorptions and malformations but at a dose level which caused maternal toxicity (LaB80).

Ethylene oxide was not embryo- or foetotoxic in rats after inhalation to a concentration of 180 mg/m^3. The only small effect observed was a slightly (but significantly) reduced foetal weight in an teratology study in male and female rats after exposure to 180 mg/m^3 (Sne82b). However, maternal toxicity was insufficiently examined. In a reproduction study of Snelling *et al.*, male foetal body weight was slightly (but significantly) reduced as well after exposure to 59 mg/m^3, but not after exposure to 180 mg/m^3 (no dose-effect relation) (Sne82a). When rats were exposed to 270 mg/m^3 ethylene oxide, prior to and during gestation, the number of resorptions was

slightly (but not significantly) increased (Hac82); in addition, a slight (but significant) effect on foetal weight was observed, but maternal toxicity was observed as well.

After a high dose of ethylene oxide (2160 mg/m^3 during 1.5 hour), teratogenic effects were observed after exposure of female mice around the time of fertilisation, but the maternal toxicity was not studied (Gen87). Post-implantation loss was observed in rats after inhalatory exposure (1800 mg/m^3) of male rats and subsequent serial mating with unexposed female rats (Emb77), however short-lasting general toxicity was observed. Finally, inhalatory exposure to ethylene oxide did not induce developmental effects in rabbits (Hac82).

In conclusion, the committee is of the opinion that the effects on the development were only observed in the absence of a dose-relationship (Sne82a) or in the presence of maternal toxicity or after exposure to high concentrations of ethylene oxide, at which maternal toxicity might be expected (Sne82b, Gen87, Emb77). The committee is of the opinion that the observed developmental effects might be secondary to maternal toxicity. However maternal toxicity is insufficiently examined. Therefore, due to a lack of appropriate data thet committee recommends not to classify ethylene oxide for developmental effects.

No data concerning the presence of ethylene oxide in human or animal milk were available.

In conclusion, a lack of appropriate data precludes the assessment of ethylene oxide for labelling for effects during lactation.

Proposed classification for fertility

Category 2, R60.

Proposed classification for developmental toxicity

A lack of appropriate data precludes the assessment of ethylene oxide for developmental effects.

Proposed labelling for effects during lactation

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

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A The committee

B Comments on the public draft

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

D Fertility and developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

The committee

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Annex **B**

Comments on the public draft

A draft of the [present reprort was released in 2001 for public review. The following persons and organisations have commented on the draft revview:

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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

interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri-postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

See next pages.

Table 1 Fertility studies with styrene.

authors	species	route	experimental period	dose	findings	remarks
Embree *et al.* (1977)	Long Evans rats	inh.	Once during 4h Males were exposed. Serial matings starting 24 h after exposure for 10 weeks with unexposed females	0, 1800 mg/m^3	A significant increase in post-implantations loss during the first 5 serial weekly matings.	Slight toxic effects which disappeared within 24 h; central depression, diarrhoea and ocular and respiratory irritation.
Snellings *et al.* (1982a)	Fischer 344 rat (males and females)	inh.	♂+♀: 6 h/d, 5 d/wk starting 12 weeks before fertilisation, 6 h/d, 7 d/wk during cohabitation (2 weeks). K: during gestation and 21 d after parturition 6 h/d, 7d/wk	0, 18, 59, 180 mg/m^3	At 180 mg/m^3: median number of pups born, median number of implantations, and the ratio of pups born to the number of implantations was significantly lower than in the control group. An increase in gestation time was also observed. No paternal or maternal toxicity was observed.	
Hacket *et al.* (1982)	Fischer 344 rat (female, n=39-41)	inh	7 hours/day; from 15 days before gestation until day 16 after gestation	0 and 270 mg/m^3	Number of resorptions was slightly increased; no other effect on reproduction was observed	Effects on development were observed at maternal toxicity levels
Mori *et al.* (1991)	Wistar rats (males)	inh.	6h/day, 5d/wk for 13 weeks	90, 180, 450 mg/m^3	In the 450 mg/m^3, epididymal but not testicular weight was reduced, and an increase in sperm head abnormalities, due mainly to the presence of immature sperm. In all dose groups an increase in malformed sperm heads (not dose-related).	

inh.: inhalation; ; h: hours; d=days; wk =weeks

Table 2.1 Developmental toxicity studies in animals with ethylene oxide.

authors	species	route	experimental period	dose	findings	remarks
Snellings *et al.* (1982a)	Fischer 344 rat (males and females)	inh.	♂+♀: 6 h/d, 5 d/wk starting 12 weeks before fertilisation, 6 h/d, 7 d/wk during cohabitation (2 weeks). ♀: during gestation and 21 d after parturition 6 h/d, 7d/wk	0, 18, 59, 180 mg/m^3	No effect on neonatal body weight except at day 21 where after exposure to 59 mg/m^3 a statistically significantly reduced male body weight was found. no maternal toxicity was observed.	
Snellings *et al.* (1982b)	Fischer 344 rat (females)	inh.	days 6 to 15 of gestation for 6 h/d	0, 18, 59, 180 mg/m^3	At 180 mg/m^3 statistically significantly reduced body weights of the foetuses and greater number of distal thoracic vertebral centra variations in ossification (not statistically significant). Maternal toxicity was not observed or examined.	
Hackett *et al.* (1982)	Sprague-Dawley rats (females)	inh.	7 h/day; 1) days 7 to 16 of gestation; 2) days 1 to 16 of gestation; 3) 15 days before gestation until day 16 of gestation	0, 270 mg/m^3	In group 3, foetal growth was reduced and the incidence of reduced ossification of skull and sternebrae were increased compared to the control (toxic effects such as decreased body weight, increased kidney and spleen weight were observed in exposed dams (group3). Number of resorptions was slightly increased.	Effects were observed at maternal toxicity level
Hackett *et al.* (1982)	New Zealand White rabbits (females)	inh.	7 h/day; 1) days 7 to 19 of gestation 2) days 1 to 19 of gestation	0, 270 mg/m^3	No reproductive toxic effects or maternal toxicity was observed.	
Embree *et al.* (1977)	Long Evans rats	inh.	Once during 4h Males were exposed. Serial matings starting 24 h after exposure for 10 weeks with unexposed females	0, 1800 mg/m^3	A significant increase in post-implantations loss during the first 5 serial weekly matings.	Slight toxic effects which disappeared within 24 h; central depression, diarrhoea and ocular and respiratory irritation.

Table 2.2 Developmental toxicity studies in animals with ethylene oxide.

authors	species	route	experimental period	dose	findings	remarks
Generoso *et al.* (1987)	(C3HxC57BL)F_1 mouse (females)	inh.	1) 1.5 h/day on four consecutive days before mating 2) 6 h/day for 10 days spread over 14 days before mating 3) 1.5 h beginning either 1, 6, 9 or 25 h after mating (mating period 30 minutes)	1) 2160 mg/m^3 2) 540 mg/m^3 3) 3240 mg/m^3	The exposure before or after mating resulted in varying increases in the incidence of mortality amongconceptuses. Exposure within 7 h after mating showed the most significant malformations. In experiments 1 and 2 maternal toxicity (not specified) was observed. For experiment 3 no detailed information with respect to maternal toxicity.	With respect to maternal toxicity no detailed information.
Generoso *et al.* (1987)	(SECxC57BL)F_1 mouse (females)	inh.	1.5 h beginning either 1, 6, 9 or 25 h after mating (mating period 30 minutes)	2160 mg/m^3	The exposure resulted in varying increases in the incidence of mortality among conceptuses. Exposure within 7 h after mating showed the most significant malformations.	No detailed information with respect to maternal toxicity.
Generoso (quoted by Sun 1986)	mouse (treated males were mated with untreated females)	inh.	4 days: 1) 6 h to 540 mg/m^3 2) 3 h to 1080 mg/m^3 3) 1.5 h to 2160 mg/m^3	cumulative dose 3240 mg/m^3	Embryonic loss occurred in group 3 six times more often than in group 1 and three times more often than in group 2 (dose response effect).	No information with respect to toxicity.
LaBorde and Kimmel (1980)	CD-1 mouse	iv	1) days 4 to 6 of gestation; 2) days 6 to 8 of gestation; 3) days 8 to 10 of gestation; 4) days 10 to 12 of gestation;	0, 75, 150 mg/kg bw (preliminary study showed that 200 mg/kg bw caused high maternal mortality)	Treatment days 8-10 or 10-12 caused a significant increase in resorptions or deads at 150 mg/kg bw. Treatment days 6-8 or 10-12 significantly increased malformations (fused vertebral arches, fused and branched ribs, scrambled sternebrae and a low incidence of exencephaly) at 150 mg/kg bw. All treatment groups decreased fetal body weight at 150 mg/kg bw.	At 75 mg/kg bw there was no maternal toxicity. At 150 mg/kg bw maternal toxicity in 1), 3) and 4); 26, 19 and 48 % mortality, respectively. Decreased weight gain during treatment in period 3 and 4.

Annex **E**

Abbreviations

Abbreviations used:

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