Diethyleneglycol (mono)alkylethers

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies 'Diethyleneglycol (mono)alkylethers'
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Mijnheer de staatssecretaris,

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

In dit kader bied ik u hierbij een advies aan over diethyleenglycol (mono)alkylethers. Dit advies is opgesteld door de Commissie Reproductietoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik wil u erop wijzen dat de commissie onder meer adviseert om diethyleenglycol (mono)methylether (DEGME) wat betreft de effecten op de ontwikkeling in categorie 2 te classificeren. Dit advies van de commissie wijkt af van het standpunt van de Europese Commissie, die DEGME in categorie 3 heeft geclassificeerd. Dit verschil in inzicht heeft echter geen gevolgen voor het opnemen van DEGME op de hierboven genoemde lijst van voor de voortplanting giftige stoffen.

Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Minister van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

prof. dr JA Knottnerus

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Diethyleneglycol (mono)alkylethers

Evaluation of the effects on reproduction, recommendation for classification

Committee for Compounds toxic to reproduction,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2003/10OSH, The Hague, 22 December 2003

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

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature and Food Quality. 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.

This report can be downloaded from www.healthcouncil.nl.

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Contents

Samenvatting

Diethyleenglycol (mono)alkylethers worden door de industrie voornamelijk als oplosmiddel. 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 voor drie diethyleenglycol (mono)alkylethers, te weten diethyleenglycol (mono) methyl ether, diethyleenglycol (mono)ethylether, diethyleenglycol (mono)n-butylether onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Diethyleenglycol (mono)methylether
  - Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat diethyleenglycol (mono)methylether de fertiliteit niet schaadt. Daarom adviseert zij diethyleenglycol (mono)methylether niet te classificeren.
  - Voor effecten op de ontwikkeling adviseert de commissie diethyleenglycol (mono)methylether te calssificeren in categorie 2 (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*) en met T;R61 te kenmerken.
  - Voor effecten tijdens lactatie adviseert de commissie om diethyleenglycol (mono)methylether niet te kenmerken wegens onvoldoende geschikte gegevens.

- Diethyleenglycol (mono)ethylether
  - Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat diethyleenglycol (mono)ethylether de fertiliteit niet schaadt. Daarom adviseert zij diethyleenglycol (mono)ethylether niet te classificeren.
  - Voor effecten op de ontwikkeling van het nageslacht meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat diethyleenglycol (mono)ethylether de ontwikkeling van het nageslacht niet schaadt. Daarom adviseert zij diethyleenglycol (mono)ethylether niet te classificeren.
  - Voor effecten tijdens lactatie adviseert de commissie om diethyleenglycol (mono)ethylether niet te kenmerken wegens onvoldoende geschikte gegevens.
- Diethyleenglycol (mono)n-buthylether
  - Voor effecten op de fertiliteit meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat diethyleenglycol (mono)n-buthylether de fertiliteit niet schaadt. Daarom adviseert zij diethyleenglycol (mono)n-buthylether niet te classificeren.
  - Voor effecten op de ontwikkeling van het nageslacht meent de commissie dat er onvoldoende geschikte humane gegevens beschikbaar zijn en dat voldoende diergegevens laten zien dat diethyleenglycol (mono)n-buthylether de ontwikkeling van het nageslacht niet schaadt. Daarom adviseert zij diethyleenglycol (mono)n-buthylether niet te classificeren.
  - Voor effecten tijdens lactatie adviseert de commissie om diethyleenglycol (mono)n-buthylether niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

Diethyleneglycol (mono)alkylethers are mainly used as solvents or intermediates. 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 the following three Diethyleneglycol (mono)alkylethers: diethyleneglycol (mono)methylether, diethyleneglycol (mono)ethylether and diethyleneglycol (mono)n-butylether.

The committees recommendations are:

- Diethyleneglycol (mono)methylether (DEGME)
  - For effects on fertility, the committee recommends not classifying DEGME on the basis of a lack of appropriate human data and sufficient animal data which show that no classification is indicated.
  - For developmental toxicity, the committee recommends classifying DEGME in category 2 (*substances which should be regarded as if they cause developmental toxicity to humans*) and labelling DEGME with T;R61.
  - For effects during lactation, the committee is of the opinion that due to a lack of appropriate data DEGME should not be labelled.

- Diethyleneglycol (mono)ethylether (DEGEE)
  - For effects on fertility, the committee recommends not classifying DEGEE on the basis of a lack of appropriate human data and sufficient animal data which show that no classification is indicated.
  - For developmental toxicity, the committee recommends not classifying DEGEE on the basis of a lack of appropriate human data and sufficient animal data which show that no classification is indicated.
  - For effects during lactation, the committee is of the opinion that due to a lack of appropriate data DEGEE should not be labelled.
- Diethyleneglycol (mono)n-butylether (DEGBE)
  - For effects on fertility, the committee recommends not classifying DEGBE on the basis of a lack of appropriate human data and sufficient animal data which show that no classification is indicated.
  - For developmental toxicity, the committee recommends not classifying DEGBE on the basis of a lack of appropriate human data and sufficient animal data which show that no classification is indicated.
  - For effects during lactation, the committee is of the opinion that due to a lack of appropriate data DEGBE 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 three Diethyleneglycol (mono)alkylethers: Diethyleneglycol (mono)methylether, Diethyleneglycol (mono)ethylether, and Diethyleneglycol (mono)n-butylether by the Health Council's Committee for compounds toxic to reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by dr J Krüse and dr JAGM van Raaij of the OpdenKamp Registration & Notification, The Hague, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evalu-

ation 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 2003, 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 online databases Toxline and Medline, starting from 1966 up 2003. 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.

Human studies on Diethyleneglycol (mono)alkylethers regarding its effects on fertility and development are described in the text. 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

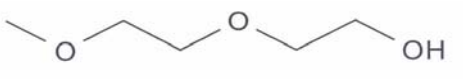
The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive 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

Diethyleneglycol (mono)methylether (DEGME)

2.1 Introduction (HCN96)

Name	:	Diethyleneglycol monomethylether
synonyms	:	2-(2-methoxyethoxy)-ethanol, Diglycol monomethyl ether, Dowanol® DM Glycol Ether, ethylene diglycol monomethyl ether, MECB, Methoxydiglycol, ß-methoxy-ß'-hydroxydiethyl ether, Methyl Carbitol® Solvent, polysolv DM
CAS-no	:	111-77-3
EC no	:	203-906-6
Examples of use	:	Solvent for coatings and ink, fuel additive,
Mol weight	:	120.15
Molecular formula	:	CH_3-(O-CH_2-CH_2)$_2$-OH
Chem formula	:	$C_5H_{12}O_3$
Structural formula	:	
Conversion factor	:	1 ppm = 5.0 mg/m^3 at 760 mm Hg and 20^{o}C 1 mg/m^3 = 0.2 ppm

The European Union has proposed not to classify DEGME with respect to the effects on fertility. For effects on development, the European Union has proposed classifying DEGME in category 3 and labeling the compound with Xn;R63.

2.2 Human studies

Fertility

No publications were found concerning the effects on fertility of DEGME in humans.

Developmental studies

No publications were found concerning the effects on development of DEGME in humans.

Lactation

No publications were found concerning the effects on lactation of DEGME in humans.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the fertility and developmental studies with DEGME in laboratory animals, respectively.

Fertility

No data were available concerning functional fertility effects.

Male and female Fischer rats (10/sex/group) were exposed subchronically by inhalation to DEGME vapour at concentrations of 0, 150, 500 or 1.080 mg/m^3 (0, 30, 100 or 216 ppm) 6 hours per day, 5 days/week, for 13 weeks (Mil85). Following exposure, all animals were weighed, sacrificed and subjected to a complete gross pathological and histopathological examination, including testis, epididymis, seminal vescicle, prostate, coagulating gland, ovary, oviduct, uterus, cervix and vagina. There were no exposure related mortalities during the course of the study. Furthermore, no apparent differences in body weights, absolute and relative organ weights between control and treated groups of animals were observed. No effects of the treatment were seen in haematology, clinical chemistry analyses, urinalyses and in the gross pathological and histopathological examinations (Mil85).

In a study of Nagano *et al*. (Nag84), DEGME (0 and 2% (~3000 mg/kg bw*) in the drinking water) was administered to male mice (JCL-ICR) for 25 days. The animals (5/group) were necropsied at the end of administration. Testicular weight and the combined weight of seminal vesicles and coagulating gland were measured. Also a white blood cell count was performed at the time of necropsy. No statistically significant differences in these parameters were noted when compared to the control group. In addition, no general toxicity was observed. The observed slight decrease in testicular weight in the treatment group was only observed in one animal.

In a subchronic study of Hobson *et al*. (Hob86), Hartley guinea pigs were dermally exposed (under occlusion) to DEGME (99% pure) at doses of 0, 40, 200 and 1000 mg/kg bw/day during 90 days (5 days/week, 6 hour/day). At the medium and high dose, the spleen weights were decreased. At the highest dose level, a statistically significant increase in serum lactate dehydrogenase (LDH) was observed. A mild fatty change in the liver was observed in all test treatment groups but not in the controls. DEGME exposure did not result in testicular lesions, nor were body weight and the relative and absolute weights of the testes, seminal vesicles and the prostate affected.

In a study by Cheever *et al*. (Che88), male Sprague-Dawley rats were treated daily with oral doses of 5.1 mmol/kg bw DEGME (613 mg/kg bw) for up to 20 days. Selected animals were killed at 2-day intervals on days 3 through 21. No gross or histopathological testicular changes were observed when compared to controls. Further, no early deaths or overt signs of toxicity were observed.

In a time course study, male Wistar rats (4-8/group) were daily administered oral doses of 2000 mg/kg bw/day DEGME by gavage for 1, 2, 5 and 20 days (Kaw90). After sacrifice, the weights of the liver, kidney, spleen, thymus, heart, lung and testis were determined. After one day, the relative thymus weight was decreased whereas the relative kidney weight was increased after 2 days. Statistically significant decreases in relative weights of the liver, spleen, thymus and testis were reported after 5 days of dosing. The decrease in thymus and testis weights was more pronounced after 20 days of treatment. In an accompanying dose-response study with oral doses of 0, 500, 1000 and 2000 mg/kg bw/day (4-8/group) during 20 days only the weights of the testis and the thymus were reported. No effect on testis weight was observed at doses of 500 and 1000 mg/kg, but at 1000 mg/kg the relative thymus weight was decreased. Animals treated with 2000 mg/kg bw/day DEGME showed a significant decrease in body weight gain (Kaw90).

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* calculated: using weight of male mice 30-35 mg, drinking 5 ml water per day.

Developmental toxicity

In an *in vivo* mouse screening bioassay for reproductive toxicity, fifty pregnant mice (CD-1) were orally administered (by gavage) 0 or 4000 mg DEGME/kg bw per day during day 6 to 13 of gestation (vaginal plug = day 0) (Sch84). There was a significant reduction in viable litters, number of live pups per litter and survival over postpartum days 1-3. Furthermore, the pup weight gain was significantly reduced. However, no routine examinations of the pups for malformations or skeletal anomalies were performed. Moreover, five dams died.

DEGME was subsequently tested in a conventional study by Hardin *et al*. (Har85, Har86), in which Sprague-Dawley rats were exposed by gavage. Doses of 0, 720, or 2165 mg/kg bw were administered per day from gestation day 7 to 16. The rats were sacrificed on day 21 of gestation. At 2165 mg/kg bw, a significant reduction of foetal body weights and number of live implantations was observed. An increasing percentage of rib variations (9.1, 42.9 and 80%) and cardiovascular malformations (0, 4.8 and 71.4%) was observed by increasing the doses. At 720 mg/kg, there was no gross evidence of foetotoxicity, although the average foetal body weight was slightly reduced. Although no maternal toxicity was observed after exposure to 720 mg/kg, a statistically significant reduction in maternal body weight (on day 21) and food consumption (first 5 days of dosing) was observed after exposure to 2165 mg/kg bw per day.

Based on the results of a dose range finding study, female New Zealand White rabbits (25/group) were dermally exposed (under occlusion) to undiluted DEGME at doses of 0, 50, 250, and 750 mg/kg bw/day from gestation days 6 to 18 (Sco86). On gestation day 29, caesarian section was performed, followed by examination of the foetuses for external, visceral, and skeletal alterations. In the highest dose group, maternal toxicity was observed, characterized by decreased weight gain (during pregnancy day 9-11) and slight haematologic changes (ie decrease in red blood cells and packed cell volume values). Two of the 25 animals in the group died. A slight (but not significant) increase in embryonic resorptions was observed. An increased incidence of developmental variations was observed in foetuses from the highest dose group as well. These foetal alterations were mild forelimb flexure, slight-to-moderate dilation of the renal pelvis, retrocaval ureter, cervical spurs and delayed ossification of the skull and sternebral bones. However, the authors were of the opinion that these effects were not severe, particularly in view of the relatively high incidence of various spontaneous malformations occurring in this species. At 250 and 50 mg/kg, no clinical signs of treatment related maternal toxicity were observed. Delayed ossification of the skull and cervical spurs were observed at 250 mg/kg. No adverse developmental effects were observed at the lowest dose (50 mg/kg) (Sco86).

In a dose-finding study, Yamano *et al*. (Yam93) exposed female Wistar rats by gavage to daily doses of 0, 125, 250, 500, 1000, 2000, 3000 and 4000 mg/kg bw/day DEGME (4-6/group). The non-pregnant rats were treated for 11 consecutive days and pregnant rats from day 7-17 of gestation. The non-pregnant rats showed a significant decrease in body weight gain and food consumption at doses above 3000 mg/kg bw and a lowering of urinary pH at all doses. Furthermore, decreased relative weights of thymus and pituitary gland, decreased white and red blood cell counts and hemoglobin concentrations and hematocrit levels were also observed at these levels. There were no signs of hepatoxicity, but at the highest dose, relative kidney weight and plasma BUN (blood urea nitrogen) levels were slightly increased, indicating weak nephrotoxicity. In pregnant rats, maternal body weight gain and food consumption were decreased above dose levels of 2000 and 3000 mg/kg bw, respectively. At 2000 mg/kg bw, the number and body weight of live fetuses were decreased, while the number of dead or resorbed fetuses increased. At higher doses, all litters were totally resorbed.

Following this dose-finding study, female Wistar rats (14/group) were administered DEGME doses of 0, 200, 600 and 1800 mg/kg bw/day by gavage from day 7 through 17 of gestation (Yam93). On day 20 of gestation, dams were sacrificed. At 600 mg/kg, dams were not affected, but foetal body weights were decreased, and foetal thymus and ossification were adversely affected. At 1800 mg/kg, maternal body weight gain, food consumption and maternal thymus weights were decreased, and visceral malformations of the cardiovascular system were observed in 28% of the foetuses. External malformations (mostly anasarca and anury) were observed in 14.1% of the foetuses at 1800 mg/kg, but not at lower doses. Dilated renal pelvis was noted in 52.8 % of the foetuses at the highest dose. Degree of ossification was considerably affected. No significant skeletal malformations were observed.

In the same experiment, eight dams per group were administered doses of 0, 200, 600 and 1800 mg DEGME/kg bw/day by gavage from day 7 through 17 of gestation. The duration of the gestation was determined and litters were examined immediately after delivery (for litter size, stillborn and live born, sex and external anomalies). On day 4 after birth, culling was performed to leave eight pups per litter. Pups were nursed by their own mothers for 21 days and pups and dams were thereupon sacrificed. In the highest dose group, the duration of gestation of prolonged by about 2 days and the number of pups was significantly decreased. The viability of the neonates was markedly affected by the treatment with DEGME and the number of live pups on day 4 after birth divided by the number of live born pups in each group were 92/100, 95/101, 58/93, and 2/37 for doses of 0, 200, 600 and 1800 mg/kg, respectively. Body weight gain of pups during 21 days after birth was unaffected at 200 mg/kg bw, but slightly decreased at a dose of 600 mg/kg bw in each sex. No significant effects of DEGME on pups were found in the skeletal observations on day 21 postnatal.

Lactation

No studies on effects on lactation were available.

2.4 Conclusions

No human data are available concerning the effects of DEGME on fertility.

Kawamoto *et al.* (1990) observed a small effect of exposure to 2000 mg/kg bw DEGME on testes weight (Kaw90). However, a decreased bodyweight gain and relative thymus weight were observed at this dose level. No effects on funtional fertility were found. Nagano *et al.* (Nag84), Miller *et al*. (Mil85) and Hobson *et al*. (Hob86) did not observe any effects on fertility after exposure to high concentrations of DEGME (up to 1000-3000 mg/kg/day) in rats, mice and guinea pigs. No general toxicity was observed as well.

Based on these results, the committee is of the opinion that a lack of appropriate human data preclude the assessment of DEGME for effects on fertility and that sufficient animal data show that no classification for effects on fertility is indicated.

No human data are available concerning the effects of DEGME on development.

Yamano *et al*. (Yam93) observed decreased foetal body weight, decreased vialibility of the pups and affected thymus and ossification in rats after exposure to 600 mg DEGME/kg bw, in the absence of maternal toxicity. After comparable oral doses (720 mg DEGME/kg bw), Hardin *et al*. (Har85, Har86) found rib variations and dilated renal pelvis in rats in the absence of maternal toxicity. Exposure to higher concentrations resulted in more pronounced developmental effects (decreased number of live pups and cardiovasular malformations), however maternal toxicity was observed as well.

In addition, Schuler *et al*. (Sch84) found a significant reduction of viable litters, number of live pups per litter, pup survival and a decreased foetal body weight in mice. However, these effects were observed in the presence of severe maternal toxicity. Based on the studies of Hardin *et al*. (Har85, Har86) and Yamano *et al*. (Yam93), the committee recommends to classify DEGME in category 2 (substances which should be regarded as if they cause developmental toxicity to humans) and to label the compound with T;R61 (may cause harm to the unborn child).

No chemical analysis for the presence of DEGME or its metabolites in human or animal milk has been performed. Moreover, no studies were available concerning the effects on lactation. Therefore, a lack of appropriate data precludes the assessment of DEGME for labelling for effects during lactation.

Proposed classification for fertility

A lack of appropriate human data preclude the assessment of DEGME for fertility and sufficient animal data show that no classification on fertility is indicated

Proposed classification for developmental toxicity

Category 2, T;R61

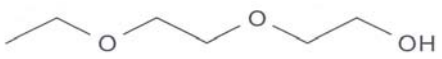
Proposed labelling for effects during lactation

A lack of appropriate data precludes the assessment of DEGME for labelling for effects during lactation

Chapter 3

Diethyleneglycol (mono)ethylether (DEGEE)

3.1 3.1.Introduction (HCN96)

Name	:	Diethyleneglycol monoethylether
synonyms	:	2-(2-ethoxyethoxy)-ethanol, APV, Carbitol®, Carbitol® cellosolve, Carbitol® Solvent, diethylen glycol ethyl ether, diglycolmonoethyl ether, dioxitol, Dowanol® DE Glycol Ether, ethoxydiglycol, ethyl carbitol, ethyl diethylen glycol, ethylene diglycol monoethyl ether, >Losungsmittel APV, polysolv, Solvosol, Transcutol.
CAS-no	:	111-90-0
EC no	:	203-919-7
Examples of use	:	Solvent for manufactoring industries
Mol weight	:	134.19
Molecular formula	:	C_2H_5-(O-CH_2-CH_2)$_2$-OH
Chem formula	:	$C_6H_{14}O_3$
Structural formula	:	
Conversion factor	:	1 ppm = 5.58 mg/m^3 at 760 mm Hg and 20^{o}C 1 mg/m^3 = 0.179 ppm

3.2 Human studies

Fertility

No publications were found concerning the effects on fertility of DEGEE in humans.

Developmental studies

No publications were found concerning the effects on development of DEGEE in humans.

Lactation

No publications were found concerning the effects on lactation of DEGEE.

3.3 Animal studies

Tables 3 and 4 (Annex D) summarize the fertility and developmental studies with DEGEE in laboratory animals, respectively.

Fertility

In a feeding study, Wistar rats (12/sex/group) were given diets containing 0.0, 0.25, 1.0 and 5.0% DEGEE (2.5 g/kg feed, 10 g/kg feed, 50 g/kg feed) for 90 days. At the highest dose, growth and food intake of both sexes were significantly reduced and one male died. Haemotological examinations, performed at week 6 and 12, showed no changes at any dose level. At autopsy, no abnormalities were noticed in gross appearance of the liver, kidneys, brain, spleen, heart, adrenals or gonads. At the highest dose level, increases were observed in the relative weights of the kidney in both sexes and of the testes. Further, histopathological examination showed hydropic degeneration in the kidneys of two males and one female, testicular oedema in five males, and a slight to moderate fatty change in the liver of most animals. No effects were observed at the lower dose levels. The test substance contained 0.4% ethylene glycol as an impurity (Hal66).

Morrissey *et al*. (Mor89) summarized the results of 48 Continuous Breeding Reproduction Studies of the NTP, one concerning DEGEE. These studies were described by Chapin *et al*. (Cha97) in more detail. The general study design and applied methods for the Reproductive Assessment by Continuous Breeding (RACB) study consists of four "Tasks". Task 1 is a 14 day dose-finding study, using six exposure groups (8 mice/

group/sex). The endpoints were clinical signs, mortality, body weight gain, and consumption of food and water. In the continuous breeding phase (Task2), a control group (n=40 animals per sex) and three treatment groups (n=20/sex) were used. Mice were exposed to DEGEE for a 7-day premating period, and thereupon randomly grouped as mating pairs. Exposure levels were 0.25, 1.25 and 2.50% in feed or water, corresponding to approximately 0.69, 3.24 and 6.20 g/kg bw /day, respectively. During cohabitation for 98 days, they were treated continuously. For all newborns during this treatment, data were collected on body weight, proportion of males, number of litters per pair and number of live and dead pups, within a period of 12 hours after birth. After the 98-day cohabitation period, the pairs were separated, but the treatment was continued. During the next 21 days, any final litters were delivered and kept for at least 21 days (weaning). If fertility is impaired in this task, a crossover mating trial of the F0 animals is performed to determine the affected sex (Task 3). Task 4 assesses the fertility and reproductive performance of the F1 generation (offspring from Task 2), which is also continuously exposed to the test substance. In Task 2, no significant effects were observed on other fertility parameters, eg fertility index, mean number of litter/ live pups per pair and sex ratio of pups born alive. In addition, an offspring assessment of reproductive function (Task 4) was performed. Therefore, the mothers were dosed through weaning and F1 mice were dosed until mated at 74 ± 10 days of age. Male offspring were mated to female offspring from the same treatment group (20/group/sex), and the F2 litters were examined for litter size, sex and pup weight. Only the highest dose level of 2.5% in feed or water (corresponding to approximately 7.1 g/kg bw/day) was tested. In this case, no effects were observed on mating/fertility index. There was a 34% reduction in the percent of sperm that were motile, however, this was observed in the presence of 12% increase in liver weight and a 6% decease in brain weight. Since no significant adverse effects on fertility were observed in the continuous breeding phase (Task 2), no crossover mating trial (Task 3) was performed (Mor89, Cha97).

The reproductive toxicity of DEGEE in CD-1 mice was assessed by Williams *et al*. (Wil90), by using a Reproductive Assessment by Continuous Breeding (RACB) protocol. Males and females (20/sex/group, 40 controls/sex) were treated with DEGEE via their drinking water resulting in dose levels of 0, 440, 2200, or 4400 mg/kg bw/day. Males and females were exposed separately during a premating period of 7 days and then continuously for 14 weeks as mating pairs. In the highest dose group the number of parental females with copulatory plugs was 84% of that in the control group (difference not statistically significant). At a dose level of 4400 mg/kg bw, female pup weights (adjusted for litter size) were reduced. DEGEE had no effect on several fertility parameters of the F0 or F1 generation mice (number of pairs with at least one litter, number of litters per pair, live pups per litter, proportion of pups born alive, or sex males/total of pups born alive), although a 34% decrease in cauda epidymal sperm motility was

observed in F1 males at 4400 mg/kg bw DEGEE. Further, adult F1 males and females showed a statistically significant increase in relative liver weight and slight decrease in brain weight at this dose level. Since the effects of DEGEE on the fertility and reproductive performance of the F0 mice during Task 2 were minimal, Task 3 was not performed.

Developmental toxicity

In an *in vivo* mouse screening bioassay for reproductive toxicity, fifty pregnant mice (CD-1) were orally administered by gavage doses of 0 and 5500 mg/kg bw/day DEGEE during days 6-13 of gestation (vaginal plug = day 0). There were no effects on the number of viable litters, live born pups per litter and weight gain, and on the percentage of pup survival from days 1 through 3 of age. Pup birth weight was slightly (but significantly) decreased. However, no routine examinations of the pups for malformations or skeletal anomalies were performed. Seven of the treated dams died (Sch84).

In an inhalation study by Nelson *et al*. (Nel84), Sprague-Dawley rats were exposed during gestation days 7-15 to concentrations of 100 ppm (558 mg/m^3) DEGEE for 7 h/day. Dams were sacrificed on day 20. Foetuses were individually weighed, and two-thirds of them were examined for soft-tissue anomalies; the other one-third were examined for skeletal defects. Data were analysed on a litter basis. No embryotoxicity, maternal toxicity, foetotoxicity or teratogenicity (visceral or skeletal defects) was seen using this study protocol.

Dermal exposure of Sprague-Dawley rats during gestation days 7-16 to 0.35 ml/day (2.6 mmol) DEGEE 4 times/day (about 6000 mg/kg bw/day) resulted in some slight skeletal effects in the foetuses, such as fused and misshapen vertebrae and centra and extra ribs. However, no increase in the total incidence of skeletal malformations was observed, nor in the incidence of visceral defects. In maternal animals, treatment weight gain and extragestational body weight gain (extragestational body weight minus day 5 body weight) were less when compared with the water control animals (Har84).

Morrissey *et al*. (Mor89) summarized the results of 48 Continuous Breeding Reproduction Studies of the NTP, one concerning DEGEE. These studies were described by Chapin *et al*. (Cha97) in more detail. The general study design and applied methods for the Reproductive Assessment by Continuous Breeding (RACB) study consists of four "Tasks". Task 1 is a 14 day dose-finding study, using six exposure groups (8 mice/group/sex). The endpoints were clinical signs, mortality, body weight gain, and consumption of food and water. In the continuous breeding phase (Task2), a control group (n=40 animals per sex) and three treatment groups (n=20/sex) were used. Mice were exposed to DEGEE for a 7-day premating period, and thereupon randomly grouped as mating pairs. Exposure levels were 0.25, 1.25 and 2.50% in feed or water, correspond-

ing to approximately 0.69, 3.24 and 6.20 g/kg bw /day, respectively. During cohabitation for 98 days, they were treated continuously. For all newborns during this treatment, data were collected on body weight, proportion of males, number of litters per pair and number of live and dead pups, within a period of 12 hours after birth. After the 98-day cohabitation period, the pairs were separated, but the treatment was continued. During the next 21 days, any final litters were delivered and kept for at least 21 days (weaning). If fertility is impaired in this task, a crossover mating trial of the F0 animals is performed to determine the affected sex (Task 3). Task 4 assesses the fertility and reproductive performance of the F1 generation (offspring from Task 2), which is also continuously exposed to the test substance. In Task 2, the mean live pup weight per litter was reduced for female pups at the highest dose level and for male pup only at the lowest dose (the mean live pup weight per litter was adjusted for the total number of live and dead pups per litter by analysis of covariance). In addition, an offspring assessment of reproductive function (Task 4) was performed. Therefore, the mothers were dosed through weaning and F1 mice were dosed until mated at 74 ± 10 days of age. Male offspring were mated to female offspring from the same treatment group (20/group/sex), and the F2 litters were examined for litter size, sex and pup weight. Only the highest dose level of 2.5% in feed or water (corresponding to approximately 7.1 g/kg bw/day) was tested. In this case, no effects were observed on mean number of live pups and mean live pup weight (Mor89, Cha97).

Lactation

No publications were available describing studies which allow for the evaluation of the effects of DEGEE on lactation.

3.4 Conclusions

No human data are available concerning the effects of DEGEE on fertility.

The effect on testes weight observed by Hall *et al*. (Hal66) in rats after exposure to DEGEE is accompanied by other toxic effects. In addition, DEGEE contained ethylene glycol as impurity which might have affected the results, since this compound is known to be a reproductive toxicant (Hal66). Furthermore, Williams *et al*. (Wil90) only found a decrease (34%) in cauda epidymal sperm motility in mice after an high dose of 4.4 g/kg bw at which an increased liverweight was observed as well.

Based on the results of Williams *et al*. (Wil90), the committee is of the opinion that a lack of appropriate human data preclude the assessment of DEGEE for effects on fertility and sufficient animal data show that no classification for effects on fertility is indicated.

No human data are available concerning the effects of DEGEE on development.

The results of an oral preliminary *in vivo* screening bioassay with mice showed a slightly (but significantly) decreased foetal body weight after exposure to DEGEE (Sch84). However, maternal death was observed as well in 7 of 50 animals. In a dermal study with rats, Hardin *et al*. found slight skeletal effects in the presence of maternal toxicity (decreased weight gain) (Har84). In an inhalation study with rats (Nel84), no effects were observed on development, however no maternal toxicity was observed as well.

The committee is therefore of the opinion that a lack of appropriate human data preclude the assessment of DEGEE for effects on development and sufficient animal data show that no classification for effects on development is indicated.

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

Proposed classification for fertility

A lack of appropriate human data preclude the assessment of DEGEE for fertility and sufficient animal data show that no classification on fertility is indicated

Proposed classification for developmental toxicity

A lack of appropriate human data preclude the assessment of DEGEE for development and sufficient animal data show that no classification on developmental toxicity is indicated

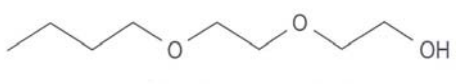
Proposed labelling for effects during lactation

A lack of appropriate data precludes the assessment of DEGEE for labelling for effects during lactation

Chapter 4

Diethyleneglycol (mono)n-butylether (DEGBE)

4.1 Introduction (HCN96)

Name	:	Diethyleneglycol (mono)n-butylether
synonyms	:	2-(2-butoxyethoxy)-ethanol, BUCB, butoxy dithylene glycol, butoxydiglycol, Butyl Carbiltol ® Solvent, O-butyl diethylene glycol, butyl dioxitol, diglycol monobutyl ether, Dowanol® DB Glycol Ether, Ektasolve DB, glycol ether DB, Jefferesol DB, poly-solv DB
CAS-no	:	112-34-5
EC no	:	203-961-6
Examples of use	:	industrial solvent, chemical and plasticizer intermediate, coalescing agent in plants, diluent for hydraulic brake fluid, solvent in household cleaners (Ema88)
Mol weight	:	162.23
Molecular formula	:	C_6H_9-(O-CH_2-CH_2)$_2$-OH
Chem formula	:	$C_8H_{18}O_3$
Structural formula	:	
Conversion factor	:	1 ppm = 6.75 mg/m^3 at 760 mm Hg and 20^{o}C 1 mg/m^3 = 0.148 ppm

4.2 Human studies

Fertility

No publications were found concerning the effects on fertility of DEGBE in humans.

Developmental studies

No publications were found concerning the effects on development of DEGBE in humans.

Lactation

No publications were found concerning the effects on lactation of DEGBE.

4.3 Animal studies

Tables 5, 6 and 7 (Annex D) summarize the fertility and developmental studies and the lactation studies with DEGBE in laboratory animals, respectively.

Fertility

In an one-generation study with rats (Charles River CD), doses of 0, 250, 500 and 1000 mg/kg bw/day DEGBE were administered by gavage to males for 60 days prior to mating and to females from 14 days before mating until day 13 of gestation, or through delivery until weaning (Nol85). Treated males were mated with untreated females and vice versa. One-half of each group of females was sacrificed on day 13 of gestation and in a uterine examination the numbers of corpora lutea, implantations, resorptions, and viable embryos were recorded. The remaining females delivered their young and the offspring were followed to weaning. Fertility of the males and females was not affected by the treatment (Nol85).

Male and female rats (Sprague-Dawley) were dermally exposed under occlusion for 13 weeks to doses of 0, 200, 600 and 2000 mg/kg bw/day (6h/day, 5 days/week) (Aul93). Histopathological examination revealed no changes in the testes, whereas vaginal cytology indicated no adverse effect on oestrus cycling. Fertility parameters, such as the male and female mating indices, pregnancy rates, male fertility indices and parturation data were not adversely affected by treatment with the highest dose (Aul93). No effects were also observed on body weight and feed consumption.

Developmental toxicity

In an *in vivo* mouse screening bioassay for developmental toxicity 48 pregnant mice (CD-1) were orally administered by gavage doses of 0, 500 or 2050 mg/kg bw/day during days 6-13 of gestation (vaginal plug = day 0). At both dose levels there were no effects on the number of viable litters, the number of live-born pups per litter, pup birth weight and weight gain, and the percentage of pup survival from days 1 to 3 of age. However, no examinations of the pups for malformations or skeletal anomalies were performed. At the low dose no maternal death or changes in body weight were observed, but at the high dose 12 of the 48 dams died (Sch84).

Pregnant Wistar rats (20/group) received doses of 0, 25, 115 and 633 mg/kg bw/day DEGBE via the diet during day 0 to 20 of gestation. Groups of 14-15 dams were killed on day 20 of gestation and the remainder (5-6) was allowed to deliver spontaneously. Pre- and post-implantation losses, number of corpora lutea, implantations and live foetuses per litter, sex ratio of live foetuses, foetal body weights and placental weight were not affected by the treatment. External, skeletal and internal examinations of the foetuses were not revealed. Only in the lowest dose group, the degree of ossification (number of caudal vertebrae) was significantly lower than in the controls. Survival of offspring up to 10 weeks of age was very high in all groups and body weight gain similar to that of the control groups. In the DEGBE-treated groups maternal weight gain during pregnancy was significantly reduced, without a decrease in food intake or any clinical sign of toxicity (Ema88).

Nolen *et al*. (Nol85) studied the effects of DEGBE in female rabbits (New Zealand White) dermally exposed from day 7-18 of gestation. Doses of 0, 100, 300 and 1000 mg/kg bw/day were applied 4h/day without occlusion. On day 29 of gestation all females were sacrificed. No significant differences were observed between the controls and the treated groups in the mean numbers of corpora lutea, implants, resorptions and viable foetuses and in the mean foetal body weight. Moreover, there were no effects on the incidence of skeletal anomalies and gross and visceral malformations. Topical application resulted in a dose-dependent mild skin irritation, but no other signs of toxicity (including maternal death) were observed during the treatment.

An additional one-generation study was performed with rats (Charles River CD) by Nolen *et al*. (Nol85). DEGBE was orally intubated at doses of 0, 250, 500, or 1000 mg/kg bw/day to male rats for 60 days prior mating and to females from 14 days prior to mating until sacrifice. Untreated males were mated with treated females and vice versa. One-half of each group of females was sacrificed on GD 13 and the uterine contents were examined. The remaining females delivered their young which were followed to weaning. No adverse effects on embryos, fetuses, or neonates were observed, except for a slight reduction of the mean pup weights during the later stages of lactation among the

offspring of the females in the highest dose group. The body weights of the females at days 1, 13, or 20 of gestation or at the weaning were not significantly different from the controls. There were no signs of maternal toxicity.

Male and female rats (Sprague-Dawley) were dermally exposed under occlusion for 13 weeks to doses of 200, 600 and 2000 mg/kg bw/day (6h/day, 5 days/week) (Aul93). Satellite groups of male and female rats were treated with 2000 mg/kg bw/day for 13 weeks, subsequently mated within their dose groups, and the females were treated through day 20 of gestation and allowed to deliver and nurse their offspring through day 21 of lactation (weaning). Pup body weights, pup survival and viability were not adversely affected by treatment with the highest dose. No effects were observed on maternal body weight and feed consumptions (Aul93).

Lactation

Male and female rats were exposed dermally under occlusion to undiluted DEGBE during 6 hr/day, 5 days/week for 13 weeks, at a dose of 2000 mg/kg bw/day (Aul93). The animals were mated within the dose groups. After mating the females were treated up to day 20 of gestation. Following delivery they nursed their offspring through day 21 of lactation (weaning). The growth and survival of pups within the treated litters was comparable to the control (Aul93).

In a one-generation study, male rats (Charles River CD) were orally dosed with 0, 250, 500, or 1000 mg/kg bw/day for 60 days prior mating and female rats to similar doses from 14 days prior to mating until weaning of the offspring (Nol85). Untreated males were bred to treated females and vice versa. The females delivered and the offspring was followed to weaning. The pups were weighed individually on days 0, 4, 7, 14 and 21 of lactation. The mean body weight of the pups from the high dose (1000 mg/kg bw) was slightly (but significantly) reduced at day 14 of lactation but not significantly at day 21 (Nol85).

4.4 Conclusion

No human data are available concerning the effects of DEGBE on fertility. No effects were observed on fertility in rats after exposure to high concentrations (up to 1000 mg/kg bw/day) DEGBE (Nol85). Moreover, Auletta *et al*. (1993) neither found any effects on fertility after dermal exposure to concentration up to 2000 mg/kg/day, nor on general body weight and feed consumption.

Based on the results, the committee is of the opinion that a lack of appropriate human data preclude the assessment of DEGBE for effects on fertility and that sufficient animal data show that no classification for effects on fertility is indicated.

No human data are available concerning the effects of DEGBE on development. Schuler *et al*. (Sch84) and Ema *et al*. (Ema88) did not find dose related effects on development, but did observe maternal toxicity (maternal death (Sch84) and reduced maternal weight gain (Ema88)). Nolen *et al*. (Nol85) studied the effects of DEGBE in rats (oral exposed) and in rabbits (dermally exposed). No developmental effects were found, however, no maternal toxicity was observed as well. Therefore, the committee considers the results of Nolen *et al*. (Nol85) negative. In conclusion, the committee is of the opinion that a lack of appropriate human data preclude the assessment of DEGBE for effects on development. The studies of Schuler *et al*. (Sch84) and Ema *et al*. (Ema88) provide sufficient animal evidence to conclude that no classification for effects on development is indicated.

In a few studies (Aul93, Nol85) the pups were exposed during lactation. In the study of Nolen *et al*. (Nol85), rats had a decrease in pup weight at the highest exposure level (1000 mg/kg bw, gavage) on day 14 after birth. However, this effect was not (significantly) present at day 21. No chemical analysis for the presence of DEGBE or its metabolites in animal (or human) milk has been performed. Therefore, the committee concludes that a lack of appropriate data precludes the assessment of DEGBE for labelling for effects during lactation.

Proposed classification for fertility

A lack of appropriate human data preclude the assessment of DEGBE for fertility and sufficient animal data show that no classification on fertility is indicated

Proposed classification for developmental toxicity

A lack of appropriate human data preclude the assessment of DEGBE for development and sufficient animal data show that no classification on development is indicated

Proposed labelling for effects during lactation

A lack of appropriate data precludes the assessment of DEGBE for labelling for effects during lactation

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A	The Committee
B	Comments on the public draft
C	Directive (93/21/EEC) of the European Community
D	Fertility and developmental toxicity studies
E	Abbreviations

Annexes

Annex **A**

The committee

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Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 2003 for public review. The following persons and organisations have commented on the draft review:

- RD Zumwalde, National Institute of Occupational Safety and Health (NIOSH), USA.
- E González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain.
- P de Kettenis, Oxygenated Solvent Producers Association (OSPA), CEFIC Sector Group, Belgium.
- E Gavini, University of Milan, Italy.
- V Foa, Department of Occupational and Environmental Health, University of Milan, Italy.

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

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

T; R60: May impair fertility

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

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

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

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

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

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

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

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

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

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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

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

Annex **D**

Fertility and developmental toxicity studies

Tabel 1 Fertility studies with diethyleneglycol (mono)methylether in animals.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproduction
Nag84	Mice (ICL/ ICR) 5/group	25 days (daily)	2.0% (oral, drinking water)	No effect on white blood cell count	No effect on testicular weight and combined weight of seminal vesicles and coagulating gland
Hob86	Guinea pigs (Hartley) 6 males/ group (control 7)	13 weeks, 5 days/ week, 6 hr/day	0, 40, 200, 1000 mg/kg bw/day (dermal under occlusion)	At 200 and 1000 mg/kg a decrease of splenic weight At 1000 mg/kg increase in serum LDH A mild fatty change in the liver in all test treatment groups.	No significant effects on weights of testes, seminal vesicles and prostate. No testicular lesions.
Che88	Rats (Sprague-Dawley) 5 males, no control group	Up to 20 days (daily); Gross and histopathological examination at 2 day intervals from days 3-21	5.1 mmol/kg bw/day (=613 mg/kg bw/day) (oral)	No early deaths, no overt signs of toxicity	No gross or histopathological testicular changes.
Kaw90	Rats (Wistar) 4-8 males/ group	Dose-response study 20 days (daily)	500, 1000, 2000 mg/kg bw/day (oral)	At 2000 mg/kg : decrease in weight gain. At 1000 and 2000 mg/kg: decrease in relative weight of thymus	At 2000 mg/kg: decrease in relative weight of testis (only the weight of the testes and the thymus were reported)

Kaw90	Rats (Wistar) 4-8 males/ group	Time-course study 1,2,5 or 20 days	2000 mg/kg bw/day (oral)	After one day decrease in relative weight of thymus After 2 days increase in relative weight of kidney After 5 days decrease in relative weight of liver, spleen and thymus After 20 days decrease in relative weight of liver and thymus	after 5 and 20 days decrease in relative weight of testis
Mil85	Rats (Fischer 344) 10/sex/ group	13 weeks, 5 days/ week, 6 hr/day	0, 150, 500, 1080 mg/m3 (inhalation)	No effects on body weights, organ weights, haemotological analyses, clinical chemistry analyses, urinalyses, and gross and histopathological examinations	No effects observed on testis, epididymus, seminal vescicle, prostate, coagulating gland, ovary, oviduct, uterus, cervix and vagina after gross pathologic and histopathologic examination.

Tabel 2 Developmental toxicity studies with diethyleneglycol (mono)methylether in animals.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproduction
Yam93	Rats (Wistar) 14 females/ group	GD 7-17 Sacrifice on GD 20.	0, 200, 600, 1800 mg/kg bw/day (gavage) from day 7-17	At 1800 mg/kg decrease of maternal body weight gain, food consumption, and thymus weight	At 1800 mg/kg: Visceral malformations of the cardiovascular system in 28% of the foetuses. External malformations (mostly anasarca and anury) in 14.1 % and
	8 females/ group	GD 7-17 Sacrifice 21 days PP		At 200 and 600 mg/kg no effects on dams	dilated renal pelvis in 52.8% of the foetuses. No significant skeletal malformations. Gestation period prolonged by 2 days. At 600 mg/kg: decrease in foetal body weight and adverse effects on thymus and ossification. At 200 mg/kg: no effects on foetuses or neonates
Sch84, Har87	Mice (CD-1) 50 females/ group	GD 6-13 (vaginal plug = day 0)	0, 4000 mg/kg bw/day (gavage)	5 dams (10%) died Maternal weight change affected	Significant reduction in number of viable litters (5/32), number of live pups, per litter, pup weight gain and pup survival over days 1-3 PP.
Har86	Rats (Sprague-Dawley) 9 females/ group	GD 7-16 , sacrifice on day 21 (dose finding study); Chernoff – Kavlock assay	0,1000, 1495, 2235, 3345, 5175 mg/kg bw/day (oral)	At 5175 mg/kg maternal mortality (2/9) and reduction of extra gestational body weight gain.	Dose related increase in visceral and skeletal malformations. At 3345 and 5175 mg/kg respectively 5/5 and 6/9 litters totally resorbed. At 2235 and 3345 mg/kg decreased foetal weight. No routine examinations of the pups for malformations or skeletal anomalies were performed.

Har86	Rats (Sprague-Dawley) 12-13 females/ group	GD 7-16 , sacrifice on day 21 Teratology study	0,720, 2165 mg/kg bw/day (oral)	No maternal toxicity. At 2165 mg/kg decreased maternal weight,	At 2165 mg/kg decreased foetal weight and litter size and increased incidence of rib and cardiovascular malformations. At 720 mg/kg increased incidence of rib malformations
Sco86	Rabbits (New Zealand White) 25 females/ group	GD 6-18 Sacrifice on GD 29 (caesarian section)	0, 50, 250, 750 mg/kg bw/day (dermal)	No treatment related clinical signs of maternal toxicity at 50 and 250 mg/kg. At 750 mg/kg decreased weight gain, concurrent physiologic decrease in red blood cells and packed cell volume valus	At 750 mg/kg: slight embryotoxicity, slight increase in embryonic resorptions In foetuses: mild forelimb flexure, slight-to-moderate dilation of the renal pelvis, retrocaval ureter,cervical spurs and delayed ossification of the skull and sternebral bones At 250 mg/kg: slight foetotoxicity (delayed ossification of the skull and cervical spurs) At 50 mg/kg: no embryonic and foetal effects

Tabel 3 Fertility studies with diethyleneglycol (mono)ethylether in animals.

Authors	Species	Experimental period/design	Dose and route	General toxicity	Effects on reproduction
Hal66	Rats (Wistar) 12/sex/group	90 days	0.25, 1 , 5 % (oral feed); test compound contaminated with 0.4% ethylene glycol	At 5%: Reduced growth rate, reduced appetite, hydropic degeneration of kidney, fatty changes in the liver.	At 5%: Increase in relative testes weight, testicular oedema
Mor89	Mice (CD-1) 20/sex/ group controls 40/sex	Exposure of breeding pairs during 14 weeks (preceeded by exposure during a premating period of 7 days) Reproductive Assessment by Continuous Breeding (RACB) protocol.	In task 2: 0, 690, 3240, 6200 mg/kg bw /day (feed or water) In task 4 only 7080 mg/kg bw /day (feed or water)		In task 2 (continuous breeding phase): At 6200 mg/kg reduction in the mean live pup weight per litter for female pups. Only at 690 mg/kg reduction in the mean live pup weight per litter for male pups. In task 4 (offspring assessment of reproductive function): No effects.
Wil90	Mice (CD-1) 20/sex/ group controls 40/sex	Exposure of breeding pairs during 14 weeks (preceeded by exposure during a premating period of 7 days) Reproductive Assessment by Continuous Breeding (RACB) protocol.	0, 440, 2200, 4400 mg/kg bw/day (drinking water)		No effects on the reproduction in the F0 and F1 generations. At 4400 mg/kg: Decrease in sperm motility. In F1 males and females increase of liver weight and decrease of brain weight.

Tabel 4 Developmental toxicity studies with diethyleneglycol (mono)ethylether in animals.

Authors	Species	Experimental period/design	Dose and route	General toxicity	effects on reproduction
Sch84, Har87	Mice (CD-1) 50 females/group	GD 6-13	55000 mg/kg bw / day (gavage)	body weight gain reduced; maternal death 14% (7/50)	No effect on viable litters, pup survival, body weight, body weight gain No routine examinations for malformations or skeletal anomalies
Har84	Rats (Sprague_Dawley) 13 females 17 controls	GD 7-16 Necropsy on day 21	0, 0.35 ml, 4 times/ day, corresponding to approximately 6000 mg/kg bw/ day (dermal)	Treatment weight gain and extragestational body weight gain s reduced.	No increase in visceral defects. Slight skeletal effects in the foetuses: Fused and misshapen vertebrae and centra and extra ribs. However, no increase in total incidence of skeletal malformations. No embryo- or foetotoxicity or teratogenicity.
Nel84	Rats (Sprague_Dawley) 15 females/ group	GD 7-15 7 hours/day Sacrifice of dams on GD 20. Foetuses individually weighed, fixed and examined for soft-tissue anomalies and skeletal defects.	0, 100 ppm (highest level that could be generated) (inhalation)	No maternal toxicity	No embryotoxicity, foetotoxicty or teratogenicity (visceral or skeletal defects)

Tabel 5 Fertility studies with diethyleneglycol (mono)buthylether in animals.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproduction
Aul93	Rats (CD) 25 /sex/group	6h/day, 5 days/week 13 weeks prior to mating and to GD 20 in females; Method used: OECD Guide line 415	200, 600, 2000 mg/ kg bw/day (dermal)	Dermal irritation; incidence, severity and, time of onset dose dependent, more severe in females; no systemic toxicity at the highest dose (2000 mg/kg bw)	No adverse effects on reproductive performance or fertility in any group. No histopathological changes in the testes observed. Vaginal cytology indicated no adverse effect on oestrous cycling
Nol85	Rats (Charles River CD) 25males/sex/ group	males: 60 days prior to mating females: 14 days prior to mating untill GD13	250, 500, 1000 mg/ kg bw/day (gavage)	No effect on body weights of the females at days 1, 13, or 20 of gestation or weaning	No effects on fertility

Tabel 6 Developmental toxicity studies with diethyleneglycol (mono)buthylether in animals.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproduction
Sch84, Har87	Mice (CD-1) 50 females/group	GD 6-13	500, 2050 mg/kg bw/day (gavage)	At 500 mg/kg no mortality, no effect on body weight.	No effect on viable litters, pup survival, body weight, body weight gain.
				At 2050 mg/kg maternal death 25% (12/48).	No routine examinations for malformations or skeletal anomalies
Nol85	Rabbits (New Zealand White) 20 females/ group	GD 7-18 (sacrifice on day 29)	100, 300, 1000 mg/kg bw/day (dermal)	Mild skin irritation at 300 and 1000 mg/kg, no mortality	No effects
Nol85	Rats (CD) 22-24 females / group	14 days to GD 13, 14 days to 21 days PP (killed day 21)	250, 500, 1000 mg/kg bw.day (oral)	No effects	No foetal effects; decrease in pup weight on day 14 PP at 1000 mg/kg bw

Tabel 7 Lactation studies with diethyleneglycol (mono)buthylether in animals.

Authors	Species	Experimental period/ design	Dose and route	General toxicity	Effects on reproduction
Nol85	Rats (CD) 22-24 females / group	14 days to GD 13, 14 days to 21 days PP (killed day 21)	250, 500, 1000 mg/kg bw/day	No effects	Decrease in pup weight on day 14 PP at 1000 mg/kg bw
Aul93	Rats (CD) 25 /sex/group	6h/day, 5 days/week 13 weeks prior to mating and to GD 20 in females	2000 mg/kg bw/day (dermal)	Dermal irritation; incidence, severity and, time of onset dose dependent, more severe in females. No systemic toxicity at 2000 mg/kg bw	Growth and survival of pups within the treated litters comparable to control
Ema88	Rats (Wistar) 5-6 females / group	GD 0-20 (killed day 20) Weaning on day 21 after birth.	25, 115, 633 mg/kg bw/day (oral)	reduced weight gain at all doses	No effects of lactation on survival rate and growth of the offspring

Annex **E**

Abbreviations

Abbreviations used:

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